A MORPHOMETRIC STUDY ON INTESTINAL MUCOSAL BIOPSIES

IN

INFLAMMATORY BOWEL DISORDERS

A DISSERTATION SUBMITTED IN PART FULFILLMENT OF THE REQUIREMENTS FOR THE M.D. DEGREE BRANCH III (PATHOLOGY) EXAMINATION OF THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY, CHENNAI TO BE HELD IN APRIL, 2016.

CERTIFICATE

This is to certify that the following dissertation bearing the title of "A MORPHOMETRIC STUDY ON INTESTINAL MUCOSAL BIOPSIES IN INFLAMMATORY BOWEL DISORDERS" is a bonafide work done by Dr. Tanush Vig in partial fulfilment of the rules and regulations for MD Branch III (Pathology) degree examination of The Tamil Nadu Dr. M.G.R Medical University to be held in April, 2016.

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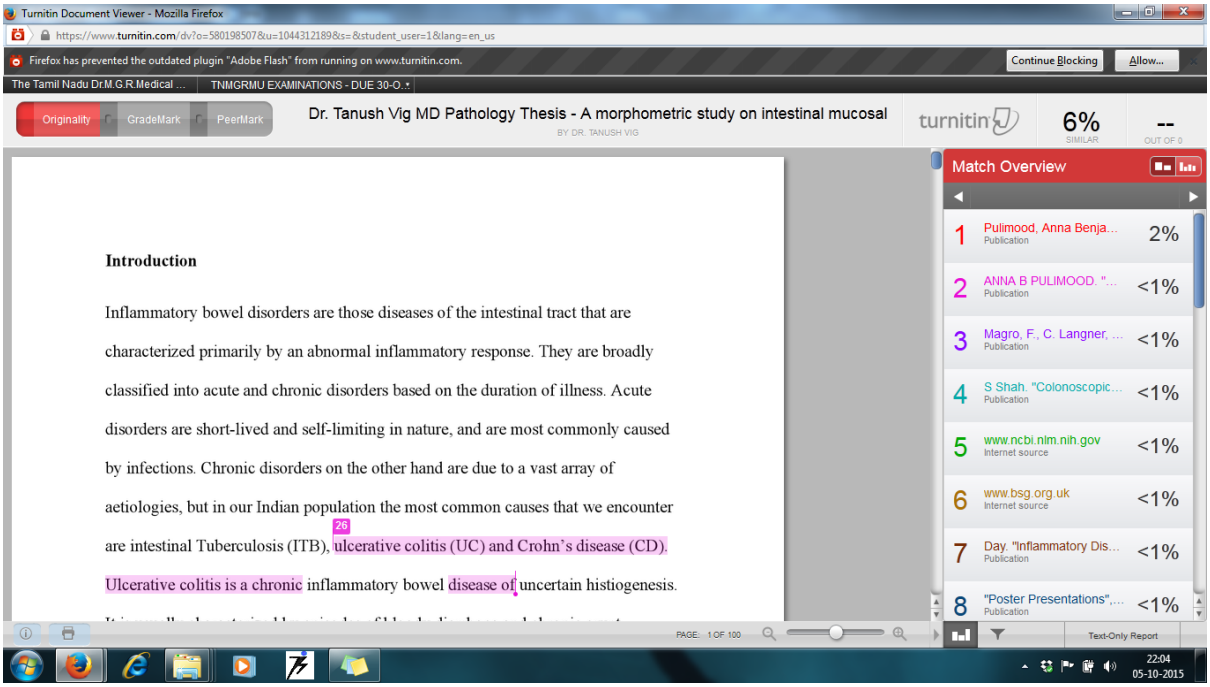
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Introduction
Inflammatory bowel disorders are those diseases of the intestinal tract that are characterized primarily by an abnormal inflammatory response. They are broadly classified into acute and chronic disorders based on the duration of illness. Acute disorders are short-lived and self-limiting in nature, and are most commonly caused by infections. Chronic disorders on the other hand are due to a vast array of aetiologies, but in our Indian population the most common causes that we encounter are intestinal Tuberculosis (ITB), ulcerative colitis (UC) and Crohn's disease (CD). Ulcerative colitis is a chronic inflammatory bowel disease of uncertain histiogenesis.
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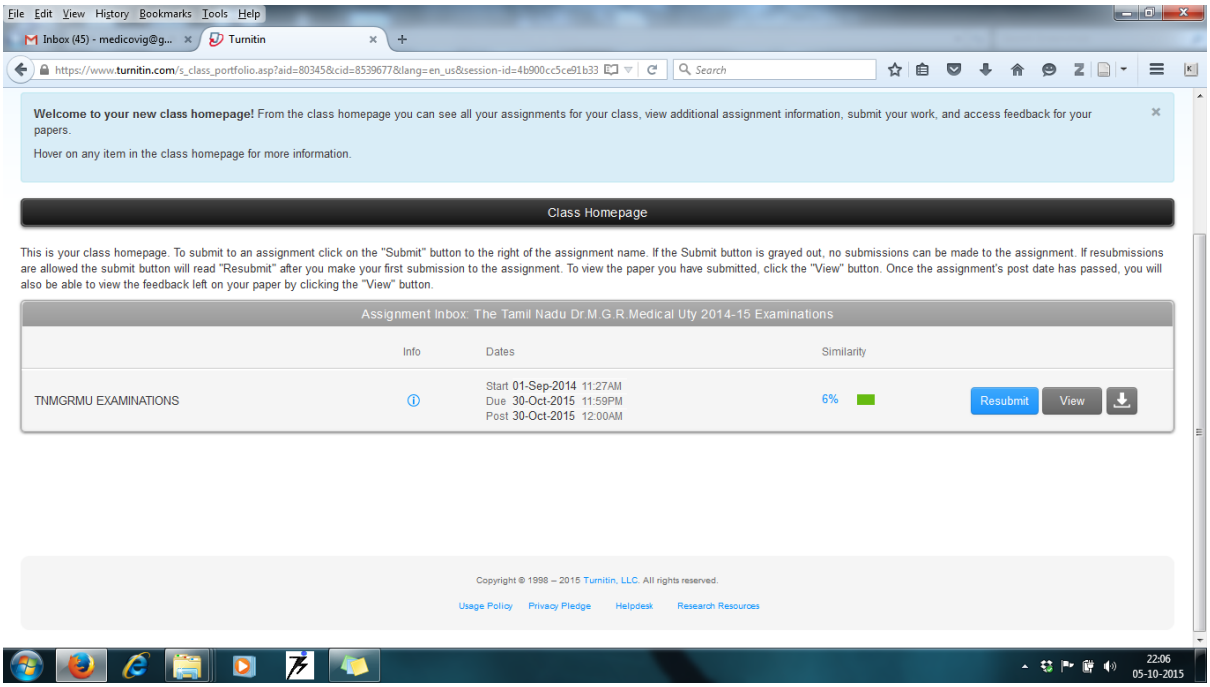
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ABBREVIATIONS

IBD	Inflammatory Bowel Disease
CD	Crohn’s Disease
ITB	Intestinal Tuberculosis
UC	Ulcerative colitis
OIF	Oil Immersion Field
HPF	High Power Field
µm	Micrometers.
AFB	Acid Fast Bacilli

CONTENTS

ABSTRACT

TITLE: A morphometric study on intestinal mucosal biopsies in inflammatory bowel disorders.

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DEGREE AND SUBJECT: M.D. Pathology

NAME OF THE GUIDE: Prof Anna B. Pulimood

OBJECTIVES: To study a few simple morphometric features and conventional existing histological parameters in ileal and segmental colorectal mucosal biopsies from patients with Crohn's disease, intestinal Tuberculosis, Ulcerative Colitis and normal controls in order to identify features that will aid in the diagnosis and differentiation of these disorders.

METHODS: We studied 30 cases each of clinically confirmed CD, ITB, UC and controls. It was a retrospective study performed on biopsies between January 2008 and January, 2015. H&E stained slides were retrieved from the archives of Dept of General Pathology. A total 480 biopsies from 120 cases were evaluated (4 biopsies from ileum, caecum/ascending colon, transverse/descending colon and sigmoid/rectum were studied in each case). 22 histological parameters were evaluated. Height of mucosa, crypts, and villi were measured in 5 well oriented fields. The mean plasma cell count of 5 consecutive OIFs and the mean eosinophil count in 5 consecutive HPFs were evaluated. All histological parameters were evaluated by 2 investigators. ICC was studied on 40 cases. Statistical analysis was performed for all variables and P-value less than 0.05 was considered significant.

RESULTS:

Controls: All biopsies were normal expect for mild chronic inflammation in caecum/ascending colon and mild architectural alteration and fibrosis in recto-sigmoid biopsies. The mean height of ileal villi and mucosa were 475.4±31µm and 569.13µm ±27.2µm. Mean height of crypt and mucosa from caecum (321.8±19µm & 333.3±21.4µm) increased to 351.53±50.19µm &

375.07±53.05µm in the rectosigmoid, respectively. The mean plasma cell count/OIF and eosinophils/HPF were 3.5, 6.6, 4.3, 3.7 and 2.3, 5.6, 3.2, 2.6 from ileum to distal segments respectively.

Crohn's disease: The percentage of involved biopsies decreased from proximal to distal segments. The prevalence of architectural alteration, chronic inflammation and activity showed the same trend. Mucosal heights were higher than controls. Mean plasma cell count ranged from 12-14/OIF in all segments and mean eosinophil count ranged from 5.3-8.7, with a peak in caecum/ascending colon. 46% of biopsies showed non-necrotizing granulomas. No case had granulomas >200µm or >4 granulomas/site.

Intestinal TB: Ileum and caecum/ascending colon were most commonly involved. Architectural alteration, chronic inflammation, activity, ulcers, fibrosis were more common and more severe than in CD in the same sites. Rectal sparing and <10 plasma cells/OIF in rectum were features in favour of ITB over CD. Granulomas >400µm, >4 granulomas/ site, deep ulceration at the site of granulomatous inflammation and rectal sparing identified 100% cases of ITB.

Ulcerative Colitis: There was a distal predominance of disease with the highest severity in all observed histological parameters. Ileum was involved in 10% cases and recto-sigmoid in 97%. UC showed a 50% increase in mucosal height compared to controls in the recto-sigmoid with a concomitant 900-1000% increase in mean plasma cell count/ OIF: highest among all diseases in our study. UC showed the highest mean percentages of crypt distortion, crypt branching and crypt abscesses. Crypt atrophy, pseudovillous change and Paneth cell metaplasia were more common in UC as compared to CD and ITB.

Key words: morphometry, IBD, Crohn disease, ulcerative colitis, intestinal tuberculosis

LITERATURE REVIEW

Introduction

Inflammatory bowel disorders are those diseases of the intestinal tract that are characterized primarily by an abnormal inflammatory response. They are broadly classified into acute and chronic disorders based on the duration of illness. Acute disorders are short-lived and self-limiting in nature, and are most commonly caused by infections. Chronic disorders on the other hand are due to a vast array of aetiologies, but in our Indian population the most common causes that we encounter are intestinal Tuberculosis (ITB), ulcerative colitis (UC) and Crohn's disease (CD). Ulcerative colitis is a chronic inflammatory bowel disease of uncertain histiogenesis. It is usually characterized by episodes of bloody diarrhoea and chronic crypt-destructive colitis involving the rectum and extending proximally. Crohn disease is a chronic, multifocal, relapsing, remitting inflammatory disease that can afflict any part of the gastrointestinal tract, from the oral cavity of foregut till the anal canal of the hindgut. It is characterized by aphthous and serpiginous ulcers, skip lesions, transmural inflammation, fibrosis and granulomas. Tuberculosis is a chronic Mycobacterial infection that most commonly affects the ileocecal region of the human gastrointestinal tract. It is associated with circumferential ulcers, strictures and granulomatous inflammation that often affects the local-regional lymph nodes.

Endoscopy and histological study of endoscopic mucosal biopsies are the most widely used techniques utilized for the diagnosis of Inflammatory Bowel Disorders. The histological diagnosis and differentiation of the Inflammatory Bowel Disorders on mucosal biopsies is based solely on identification of the degree of architectural

alteration, chronic inflammation and granulomas, when present, in the mucosa and superficial submucosa. Since the intestinal mucosa is normally infiltrated by various inflammatory cells, the definition of chronic inflammation is based on identifying an increase in the number of inflammatory cells in the mucosal lamina propria. Similarly, the definition of architectural alteration significant enough to warrant a diagnosis of Inflammatory Bowel Disease is based on identifying an abnormal distortion of normal mucosal structures. Since normal intestinal mucosa may show minor architectural alterations, the pathologist must be aware of the acceptable limits of abnormality in the normal mucosa.

It is widely accepted that the intestinal mucosa of residents of tropical countries may show mild architectural alterations and an increased number of inflammatory cells in the small intestinal mucosa, even in the absence of significant specific inflammatory bowel disorders. These alterations have been termed tropical enteropathy and their aetiology is believed to be related to the luminal bacterial content, among other dietary and host environmental factors. All morphometric studies on the intestine have been done only on biopsies from the proximal part of the small intestine (duodenum or jejunum) and occasional studies have utilised rectal biopsies only and no data is available on the normal architecture and inflammatory infiltrate in the tropics of the ileum and colon, which are the sites commonly biopsied for the diagnosis of Inflammatory Bowel Disorders.

Epidemiology of Inflammatory Bowel Disorders

Inflammatory bowel disease (IBD) is traditionally considered to be a disease of the western population. But as new studies emerge and publish new results it is evident that IBD is on a rise in the eastern side of the world now[1–3]. There is a significant variation in the geographical incidence of IBD. North America and Europe report the highest incidence whereas the incidence is lower in South America, the African subcontinent and the Asia-Pacific region. As globalisation, industrialisation and urbanisation have spread drastically to the East, the incidence of IBD has increased accordingly. As the incidence of IBD paralleled the industrialisation, development and urbanisation, investigators have labelled them as potential risk. These could also be attributed to the increased awareness of the disease and the better facilities available for the diagnosis of IBD[1,4]. In India, UC is more common and has a higher prevalence than CD. According to a recent study published from India by Sood et al they reported an incidence of 6.02/ 1 lakh and prevalence of 44.3/ 1 lakh for ulcerative colitis[5]. Their data represents figures comparable with the data reported from the western studies[4]. Although the prevalence of CD has been increasing worldwide, it was believed to be rare in developing countries and the Indian subcontinent where tuberculosis is more prevalent, However it is now well documented and accepted that Crohn's disease occurs in India [2,4,6–11]. However, the exact "true" figures to represent its incidence and prevalence are unavailable as large scale population based studies are necessary. Developing countries in the east have traditionally been burdened by Tuberculosis however, TB is currently on the surge even in western countries as a direct result of to trans-migration of population around the globe and the notorious

HIV virus causing immunosuppression[12,13]. Thus, in India increasing incidence and prevalence of IBD coupled with the ever prevalent Tuberculosis poses a difficult scenario for gastroenterologists and pathologists since these diseases are chronic in nature and have significant clinical and histo-pathological overlap [7,9,14–20].

Pathogenesis

Intestinal Tuberculosis (ITB) is caused by *Mycobacterium tuberculosis*, an aerobic, non-motile, acid fast bacillus, identified and described on 24 March 1882 by Dr. Robert Koch, for which he was awarded with the Nobel prize in 1905. According to the fact sheet of WHO approximately 33% of the human population is infected and harbour M. Tuberculosis while new infections occur in approximately 1% of the population every year. Tubercle bacilli enters the intestinal tract in patients after they cough and swallow sputum with the bacilli. The bacilli cause disease and pathology preferentially in the vicinity of the ileo-caecal junction owing to the slow motility in this region due to the function of the ileo-caecal junction. Microbiological demonstration via special stains and/or culture remains the gold standard for diagnosis.

The histiogenesis of IBD is still unknown however, recent advances have shed light on the possible mechanisms about their activation and disease progression. Recent literature suggests that IBD may result due to an uncontrolled inflammatory response to intestinal microorganisms in a host that is genetically susceptible [21]. Genes suspected to play a role in the aetiopathogenesis of IBD include the nucleotide oligomerization domain 2 (NOD2), autophagy genes, and components of the

interleukin-23–type 17 helper T-cell (Th17) pathway [21–24].ATG16L1, is an autophagy gene and has been found to be associated with Crohn's disease but not with UC so far. The interleukin-23–Th17pathway controls microbial defence and intestinal inflammation. Multiple genes regulating this pathway have been found to be associated with Crohn's disease and ulcerative colitis [24]. However, further research is still needed to separate these 2 closely mimicking entities at a molecular and genetic level.

Clinical features

Both TB and CD share a set of similar clinical features. Fever, weight loss, malabsorption, diarrhoea and haematochezia are see in both of these diseases. As both these diseases can involve the intestinal wall up to the serosal aspect, abdominal pain and vomiting accompanied with abdominal distension are seen in both. As disease progresses the luminal compromise may result in intestinal perforation and intra-abdominal abscess formation or peritonitis. Features of peri-anal fistulas also can occur in both. These features are sharply different from UC that involves the mucosa limited to the length of the colon. Patients with TB usually have a history of contact with a case of pulmonary TB and also have involvement of lungs, pleura and lymph nodes [9,25]. Patients with CD and UC are more commonly associated with extra-intestinal manifestations like spondylitis, uveitis, arthritis, etc. Primary sclerosing cholangitis is more commonly associated with UC than CD. It has been found that the duration of illness in most cases of CD is more than 1 year while it is relatively shorter in cases of intestinal tuberculosis[9,25]. Individuals with CD and UC commonly

complain of watery diarrhoea and haematochezia. Disease limited to the peri-anal region relapses frequently after surgery favours a diagnosis of CD over UC and over intestinal TB[7]

Endoscopic features:

Both upper and lower GI endoscopy can provide crucial information to help in differentiating the inflammatory bowel disorders. The frequency of endoscopic involvement of different sites is similar in ITB and CD except for the rectum that is generally not involved in ITB[26]. Many of the changes caused by acute inflammation of the colonic mucosa - namely, oedematous mucosal folds, ulceration of the mucosa and nodularity, luminal narrowing, strictures and pseudopolyps, can occur in both conditions[27]. One of the most important distinguishing feature is that the ulcers in Crohn's disease occur on a normal appearing mucosa, whereas in tuberculous colitis the mucosa surrounding the ulcer has reactive mucosal changes of inflammation such as erythema, nodularity or oedema[14,28–30]. Individual endoscopic features of both CD and intestinal ITB have been described , however not a lot of studies have compared or tried to differentiate between intestinal TB and CD solely on endoscopic grounds [14].

Although a cobblestone appearance of the mucosa has been reported in tuberculosis of the colon its presence is strongly suggestive of Crohn's disease. Colonic strictures caused by tuberculosis are generally but not invariably short and <3 cm. Mucosal bridging has not been described in tuberculous colitis. Skip lesions and longitudinal, deep, fissuring ulcers are significantly more frequent in patients with CD. Transverse ulcers are classically observed in intestinal TB [4,9,19,31,32]. In a recent prospective study

from India, Makharia et al[33] found that skip lesions in the colon were significantly more frequent in patients with CD compared to patients with intestinal TB (66%*vs*17%)as were aphthous ulceration (54% *vs.* 13%),linear ulceration (30% *vs.* 7%) and superficial ulceration(51% *vs.* 17%). Cobble stone appearance of the colonic mucosa was seen only in CD (17% *vs.* 0%). Nodularity of the colonic mucosa was significantly more common in patients with ITB than in those with CD (49% *vs.* 24.5%). An endoscopic study dedicated to differentiate between ITB and CD in the Korean population[14] proposed 4 features in favour a diagnosis of intestinal TB and 4 features in favour of a diagnosis of CD. However, further studies are necessary to confirm their proposal before utilising them for routine diagnostic purposes.

Ulcerative colitis differs from these 2 disorders as it classically affects the entire length of the colon with a diffuse involvement with ulceration, erythema pseudopolyps and usually does not show features of skip-lesions or cobble stoning. UC shows a distal predominance of the disease and usually the rectum and sigmoid colon bear the maximum brunt of the disease[34,35]. However, certain studies show that some cases of UC in adults and paediatric population can show skip lesions and discontinuous involvement of the colon, adding to the diagnostic dilemma[36–38]. Hence endoscopic features in isolation without histological examination of segmental colo-rectal mucosal biopsies cannot subtype IBD or separate IBD from acute self limiting colitides.

PATHOLOGY OF UC, CD AND ITB

Ulcerative colitis (UC)

On gross examination of a resected specimen of UC conventionally we find diffuse chronic inflammation that is continuous in nature without any intervening areas of normal appearing mucosa. The disease involves the rectum predominantly and then the inflammation and pathology spreads proximally towards the ileo-caecal junction. During its proximal spread the disease gradually also tapers in its severity. Usually this transition area between the normal and the diseased mucosa is well circumscribed and sharp [35]. The mucosa shows superficial ulceration and has a granular and friable texture[39,40]. According to the extent of disease, UC can be divided into ulcerative proctitis, left-side colitis, sub-total colitis, and pancolitis[41].Whenever there is extensive ulceration, the remaining normal mucosal islands may result in pseudopolyps which are essentially the superficial regenerating mucosa attaining a polypoidal appearance. These are relatively common in the sigmoid and descending colon and uncommon in the rectum[42] . Unusual inflammation patterns are rectal sparing, caecal patch and backwash-ileitis. Studies by different investigators have reported that rectal sparing can involve 30% of untreated cases, 13% in fulminant cases of UC and 44% of cases on treatment [42–46]. The term backwash ileitis refers to those cases of UC in which ileitis occurs secondary to diffuse severe colitis. It is estimated to occur in 20% of cases with diffuse colitis[47]. In this scenario differentiation from CD can be a challenge. Mucosal damage leads to subsequent repair with fibrosis which, in contrast to CD, is limited to the mucosa[48].

UC is a chronic disease that results in inflammation restricted to the mucosa accompanied with significant distortion of the normal mucosal architecture. Architectural alteration and abnormal pseudo-villous architecture are more common in UC as compared to CD (25-70% vs. 12%) [49–52]. Unlike CD, UC does not show fissures and transmural inflammation apart from rare cases with fulminant colitis [52].

The inflammation is continuous without any skip lesions and classically tapers in severity as we move proximally from the rectum. In the active state neutrophils are seen within the lamina propria and can result in cryptitis and crypt abscess formation[16,45,53–55]. The presence of crypt abscess has been found to be statistically more common in UC as compared to CD (41% in UC vs. 19%) [49].

"Basal plasmacytosis" as defined by the presence of plasma cells between the muscularis mucosa and the base of crypts has been regarded as a strong discriminator of IBD from non-IBD colitis. However studies show that the presence of basal plasmacytosis cannot differentiate between UC and CD[50,56,57].

Crohn's disease (CD)

CD can involve any segment of the GI tract, right from the oral cavity till the anal canal. Terminal ileum accompanied with proximal right colon is the most common site to be involved in the vast majority of cases. Again it is not uncommon to have isolated large bowel CD (20% cases) in association with disease involving other sites[16]. CD is characterised by deep fissuring ulcers, intra-abdominal abscesses , blind

sinuses and stricture formation in chronic cases [17,29,53,58]. As classically described by Morson and Lockhart-Mummery, the resected segment of bowel will show a discontinuous pattern of involvement. Diseased areas are admixed with uninvolved areas of normal bowel leading to the rise of "skip-lesions". There is abrupt transition from uninvolved areas to the diseased areas. The mucosa is oedematous and congested. In CD the pathology extends transmurally and hence, the serosa may show adhesions or serosal exudates. This leads to a feature called "fat wrapping" described as adipose tissue of the mesentery expanding towards the anti-mesenteric region that is normally free of fat. Sheehan et al[59]have shown that fat wrapping carries a high predictive value in diagnosis of CD as among the 225 small intestinal resections that they studied fat-wrapping was seen only in cases of Crohn disease. Fat-wrapping represents part of the connective tissue changes that are encountered in Crohn disease. The earliest grossly visible mucosal lesions of CD are small aphthous ulcers that typically develop over lymphoid follicles. As the aphthous ulcers enlarge, they coalesce to form larger serpiginous ulcers with heaped-up oedematous margins[52]. Islands of oedematous, non-ulcerated mucosa, separated by deep discrete ulcers may give rise to the classic cobblestone appearance[17]. Grossly identifiable features like strictures, fissures, fistulous tracts and even free perforation are more commonly seen in CD as compared with UC [60]. As disease becomes chronic, stricture formation occurs at the sites of transmural inflammation and damage with subsequent fibrosis leading to thickening of the bowel wall.

Histologically, CD is associated with chronic inflammation and architectural changes in the mucosa as in UC, though the changes tend to be patchy or focal. Architectural

alteration, cryptitis, crypt abscesses, crypt distortion and basal plasmacytosis, all features seen in ulcerative colitis are also seen in CD. Another characteristic feature of Crohn's is the presence of characteristic granulomas composed of epithelioid histiocytes that separate them from UC. These granulomas are classically non-caseating in nature, infrequent and small. Multinucleate giant cells are not a usual feature in CD. Granulomas are seen in approximately 50%-60% of resection specimens of CD [7,16,19,33,55]. There has been some debate about the nature of pericryptal granulomas as Surawicz et al reported that peri-cryptal granulomas are related to mucin extravasation by crypt damage and is not a reliable feature of CD However, a later study by Lee et el showed that none of the pericryptal granulomas had any evidence of mucin demonstration by the use of special stains [61]. They also went on to summarize that pericryptal granulomas found in isolation are more likely to be found in cases of Crohn's disease. Another feature of note in resected specimens is the presence of transmural lymphoid aggregates referred to as transmural lymphoid hyperplasia, characteristically located away from areas with ulceration. In one study of colectomy specimens the investigators found that the presence of granulomas and transmural lymphoid hyperplasia appeared to be the 2 most specific differentiating features between cases of fulminant colitis and CD[43]. Pyloric gland metaplasia is a feature of chronicity of inflammation commonly seen in mucosal damage, recurrent ulceration and healing. It is seen in 2-27% of CD cases and is particularly common in the ileum. Though studies have shown that pyloric gland metaplasia can rarely be seen in resection specimens of UC, this feature has not been reported in ileal biopsies of cases with UC[62,63].

Intestinal Tuberculosis (ITB)

ITB classically causes ulceration, short strictures and marked thickening of the bowel wall due to inflammation, fibrosis and adhesions, or a combination of these. The ulcers are transverse, often circumferential, with ill-defined, sloping or overhanging edges. The surrounding mucosa may show flattening of folds, ulcers, erosions and pseudopolyps. The cut section of the intestinal wall shows scarring and necrosis, often with loss of distinction of the different layers. The serosal surface may show 2-5 mm sized nodules and adhesions. The regional lymph nodes are invariably enlarged and may show caseation[28].

The histological hallmarks of ITB are confluent, caseating granulomas containing acid fast bacilli and surrounded by a lymphoid cuff. These are found in all layers of the intestinal wall and in regional lymph nodes, but sometimes only in the latter [28]. Early granulomas are usually found within lymphoid tissue [64]. There may be extensive pyloric metaplasia. Occasional superficial fissuring ulcers that extend into the submucosa may be seen. Healing occurs by fibrosis and epithelial regeneration begins at the edge of ulcers. Healing granulomas are surrounded by a rim of fibrous tissue in lymph nodes, but not in the intestinal wall [28].

Macroscopically, ITB and CD both can show bowel wall thickening, skip lesions and strictures, but the latter are longer in CD than in ITB.

Microscopically, common features are aphthous ulcers over lymphoid follicles, fissuring ulcers that extend into the muscularis propria or deeper, distortion of the

mucosal architecture, pyloric gland metaplasia, cryptitis and crypt abscess formation with moderate to severe chronic inflammation. These changes are often segmental or patchy and extend transmurally.

Definitions of histological parameters

As histological examination is subjective and inter-observer variability inevitably occurs, many investigators have worked on trying to establish uniformity in reporting and evaluation of biopsies. Many of them have attempted to also define certain histological parameters in order to increase their reproducibility by others for research and diagnostic purposes. The following are definitions of various histological parameters used in the diagnosis of IBD.

Crypt distortion: Any deviation from the normal crypt architecture. May include branching of crypts (vertical or horizontal), loss of parallelism and normal "test-tube appearance". Also included are crypt irregularity, dilatation and significant variation in shape and size of individual crypts. Care must be taken when observing crypts located adjacent to lymphoid follicles and anal transition zone as these are found to be abnormal in normal healthy controls too[65–72].

Crypt branching: The normal rectal mucosa in the regions of innominate folds shows crypt branching at the luminal surface. The presence of at least 2 or more branched crypts in a well-oriented biopsy is considered as adequate evidence of crypt branching

and is considered abnormal. This histological parameter is included in crypt distortion[45,50,51,66–68].

Crypt atrophy: Significant crypt shortening with an increased gap between the base of crypts and the superior part of muscularis mucosa is regarded as abnormal. Another additional feature apart from crypt shortening, is the presence of wide gaps between consecutive crypts. Some authors advocate more than 1 crypt diameter space between crypts as evidence of atrophy[49,51,65,68,69,73,74].

Villiform/irregular mucosal surface: An undulating or a small intestinal villi-like appearance of a colonic mucosal biopsy with or without broad crypt mouths. This feature is also referred to as pseudo-villous change [56,65].

Basal plasmacytosis: Regarded as the presence of plasma cells at the base of the mucosa. It may be seen separating the crypt base from the muscularis mucosa, however, it may not always be sub-cryptal. This feature is appreciated along with trans-mucosal inflammation. Schumacher et al describes this change as crypts with their “feet” dipping in a pool of plasma cells. The presence of basal plasma cells is normal for the caecum and ascending colon, but the plasma cell gradient is still maintained [45,65,66,75,76].

Loss of plasma cell gradient: It is found that the number of plasma cell is normally more in the upper part of the mucosa and this quantity tapers in number as we move inferiorly towards the lowermost part of the mucosa. Studies have confirmed that the

inflammatory content, including plasma cells, in the upper part of the mucosa is 2 times that of the lower half [54,77,78].

Cryptitis: The presence of neutrophils within the lining epithelium of crypts[50,66,70,79].

Crypt abscess: The presence of neutrophils within the lumen of a crypt, often seen closer to the base of crypts[69,70,73,74].

Granulomas: An epithelioid cell granuloma is defined as a discrete collection of at least five epithelioid cells (activated histiocytes with a homogeneous pale eosinophilic cytoplasm and elongated slipper-shaped nuclei). Based on size, granulomas are classified as small (<200 microns), medium (200-400microns) and large (> 400microns). Granulomas may or may not contain multi-nucleate giant cells and can be further sub-classified into necrotising and non-necrotising [7,20,33,51,68,73].

Diagnosis of Inflammatory Bowel Disorders and the role of mucosal biopsies

The most important tools in the diagnosis and differentiation of intestinal inflammatory disorders are endoscopy and mucosal biopsies, although clinical features, radiology, other laboratory and molecular investigations are all useful. One of the drawbacks of conventional histological evaluation is that it is subjective, and dependent on the experience of the individual pathologist[780]. Unfortunately there is also considerable overlap in the pathological changes of the different inflammatory disorders, and distinguishing them is often difficult. A mistaken diagnosis can result in considerable and prolonged morbidity. For instance, the histological changes of CD

and UC are very similar, but differentiation is crucial since their surgical management is entirely different. The histological changes in intestinal tuberculosis (TB) and CD are also very similar, but their medical management is completely different. The administration of immunosuppressant induces healing in CD but can precipitate rapid and sometimes fatal progression of intestinal TB[79]. Improving the diagnostic accuracy of mucosal biopsies, therefore, would be of great value to both clinicians and patients.

Histological features that can help in differentiating the diseases from one another have been described but again, no single feature is virtually pathognomonic or diagnostic except for caseation and the presence of Acid fast bacilli in tuberculosis. Sometimes most of the discriminating histological features might not be present in the biopsy submitted due to sampling error. A minimum number of histological features required for a particular diagnosis has also not been defined. Thus to arrive at a correct diagnosis the constellation of histological features must be taken together along with the clinical details and endoscopic findings and any other adjunctive investigation, if available.

Another limitation of histological examination of mucosal biopsies is that epithelioid cell granulomas that serve as the primary differentiating feature to distinguish between Crohn disease and intestinal tuberculosis are found in only 50-80% of mucosal biopsies from clinically confirmed cases of TB and 15-65% of clinically confirmed

cases of CD [7,32,33,81]. Caseous necrosis and demonstration of acid fast bacilli by special stain is found in only 18-33% of cases and around 5% of cases respectively [7,31,32]. Pulimood et al have reported that the presence of confluent granulomas, prominent lymphoid cuff around granulomas, epithelioid histiocytes in the base of ulcers, more than 4 granulomas in one segment and large granulomas (more than 400 microns in dimension) were statistically significant in diagnosing cases with intestinal tuberculosis [7,19,20]. Features reported to favour CD in mucosal biopsies are less than 5 granulomas per segment, small granulomas less than 200 microns in dimension and discrete, poorly organised granulomas. Microgranulomas and pericryptal granulomas are also features in favour of CD[19,20,30].

Many studies have shown that there are certain histological features that are reliable in differentiating IBD from acute self limiting colitis and sub classifying IBD into UC and CD [45,50,57,60,65,71,75,76,82–86].

Features found to be reliable in favouring IBD over infective/ acute self-limiting colitis are

1. Basal plasmacytosis.
2. Crypt distortion/ crypt/ branching/ abnormal crypt architecture.
3. Crypt atrophy
4. Irregular/ villous mucosal surface.

Features that favour infective colitis over IBD are – preserved crypt architecture with the absence of features of IBD including the absence of any branched crypts, or basal plasmacytosis.

Features found to be reliable in favouring a diagnosis of UC over CD are :

1. Diffuse crypt distortion between sites.
2. Crypt atrophy.
3. Villous/ irregular mucosal surface.
4. Mucin depletion.
5. Absence of ileal inflammation.
6. Absence of features in favour of Crohn's.

Features found to be reliable in favouring a diagnosis of CD over UC are:

1. Granulomas not associated with crypt-rupture.
2. Focal / patchy chronic inflammation in the lamina propria.
3. Focal/ segmental crypt distortion.
4. Involvement of the ileum.
5. Absence of features in favour of ulcerative colitis.

Problems in the diagnosis of UC, CD and TB

Many features of UC and CD can overlap which can lead to an erroneous diagnosis. The features of CD also overlap with those of intestinal TB in terms of clinical, radiological, endoscopic and histological parameters, and it can be difficult to arrive at a correct diagnosis for both experienced pathologists and gastroenterologists. In our country where Tuberculosis is highly prevalent and now when Crohn's disease is on the rise, it has become increasingly more important to make a correct diagnosis [1911]. In case of misdiagnosis of TB, unnecessary initiation of anti-tubercular medication poses the risk of toxicity and treatment of the actual underlying disease (CD) is delayed leading to prolonged morbidity. Treatment with steroids for Crohn's disease can be disastrous if the diagnosis of Intestinal Tuberculosis is missed [9].

A review of the files of the department of G.I. Sciences at CMC, Vellore between 1990 and 2004 showed that 1232 new cases of chronic inflammatory bowel diseases were diagnosed on lower gastrointestinal mucosal biopsies during that period. 669 (54.3%) were diagnosed as UC, 256 (20.7%) as CD and 94(7.6%) were classified as inflammatory bowel disease, but could not be further categorized. Of 373 cases with granulomatous inflammation, 160 (43%) were diagnosed to have intestinal TB and 147 (39%) as CD. 53 biopsies (14%) with granulomatous inflammation could not be further categorized as TB or CD (AP, PhD Thesis 2010).

Differentiating between IBD and other colitides can sometimes also be difficult as a result of shared and overlapping features. Branched crypts were seen in 30% of acute self limiting colitis in one study [74] and Surawicz et al reported 10% in their study [57].

Pseudovillous change can be seen in upto 7% of non-IBD cases[50,74,76]. Crypt atrophy, architectural alteration, basal plasmacytosis, pericryptal granulomas, cryptitis and crypt abscesses have also been reported to be present in 15%, 30% ,6, 15%, 47 and 54% of non-IBD cases respectively [50,56,66,69,71,79,84–86].

This highlights the magnitude of the problem of differentiating between these different disorders and the need for better, more accurate, histological parameters.

Normal mucosa and tropical enteropathy

The terminal ileum has villi that are approximately 3 times the height of crypts. Lymphoid aggregates or Peyer's patches are common in the terminal ileum. The lamina propria has a few plasma cells, lymphocytes, macrophages and eosinophils.

The normal colonic mucosa has crypts that are relatively straight and distributed evenly. The lowest end of the crypts is close to the muscularis mucosa. The lamina propria of the normal colonic mucosa has lymphocytes, plasma cells and smaller numbers of eosinophils. The density of these cells may vary between different segments of the colon, with decreasing numbers from the caecum to the rectum. Within the mucosa, the superficial mucosa has up to twice the number of inflammatory cells as the deep mucosa[54,82].

The intestinal mucosa of individuals from tropical parts of the world has been shown to exhibit varying degrees of damage of the small intestinal mucosa and there is some evidence that this is also seen in the colon. Migrants from the tropics to temperate

regions show improvement in their small intestinal morphology that reverses on visiting their country of origin (21). Factors thought to contribute to these changes include infections and malnutrition(22) and the changes in mucosal architecture correlate with environmental hygiene and evidence of parasitic infection on stool examination(23). Bacterial overgrowth in the proximal small intestine has also been postulated to be one of the causes. The mucosal abnormalities in tropical enteropathy include widening of villi, an increase in the height of crypts and an increased mononuclear cell infiltrate in the villous epithelium and lamina propria. The mucosal changes have not been quantitated and could potentially pose a problem in the diagnosis of Inflammatory Bowel Disorders.

Morphometry in the diagnosis of inflammatory bowel disorders

Histological diagnosis requires appreciation of features in a biopsy to suggest differentiation of IBD from non-IBD and for subtyping of IBD in to UC and CD. However, no feature is virtually pathognomic and many of the salient features that help usin diagnosis might not be present in the biopsy sample. Another problem is the inter-observer agreement between investigators to agree upon a particular feature as histological assessment is highly subjective. Morphometric analysis on the other hand is an objective measurement. This makes it more reproducible and reliable than simple subjective assessment.

As a result of various studies few reliable histological parameters have emerged that are associated with a high inter-observer agreement. Based on these results, some investigators have tried to analyse these features along with the other histological parameters that we pathologists use in our daily practice to see if morphometric analysis of biopsies can provide consistent results and improve diagnostic capabilities.

The most important mucosal changes that occur in inflammatory disorders of the intestines are architectural alteration of crypts and villi and chronic inflammation in the lamina propria. The distribution of chronic inflammation in the colonic segments, degree of architectural alteration, activity and the presence of granulomas unrelated to crypt rupture are useful in distinguishing CD from UC[16,49,51,61,90,91]. Subjective evaluation of mucosal inflammation and architectural alteration have also shown some difference in their distribution in intestinal TB and CD [9219]. Studies have shown that basal plasmacytosis is one of the most reliable feature for diagnosing IBD, but as it is seen in both UC and CD, this feature cannot help in differentiating between the two. Basal plasmacytosis has conventionally been evaluated on a low power examination. A few studies have counted plasma cells in the lamina propria, some using Immunofluorescence and immunohistochemistry to subtype the immunoglobulin secreting plasma cells into IgA, IgG and IgM types [54,77,78,93–95]

A study by Rosekrans et al[77] showed that there was a marked increase in the plasma cell counts in cases of IBD as compared with the normal controls. In both rectal and sigmoid mucosal biopsies, counts of IgM plasma cells are higher in CD as compared to UC. Counts of IgG plasma cells, on the other hand, are higher in UC as compared

to CD. Scott et al [94] showed that plasma cells counts in the lamina propria were almost similar in their study and could not differentiate among UC and CD, although they were higher in cases of IBD as compared to normal controls and non-specific colitides. JM Skinner et al[78] used Immunofluorescence technique to count plasma cells in their study and showed that it was only IgA-subtype of plasma cell that showed a statistically significant difference between CD and UC (4735 Vs 2555). These studies differ among each other in the methodology used and in the representation of their data. Moreover, these studies have counted plasma cells in the lamina propria on low power examination. We know that in IBD there is a marked increase in the cellularity of the deep lamina propria, including the population of plasma cells, but no study has quantitated the number of plasma cells exclusively in the lowermost part of the mucosa, in the sub-cryptal and inter-cryptal area. Also these studies are slightly complex requiring procedures and software that are not feasible for routine diagnostic practice by a pathologist. Also interesting to note is that no data is available from the Indian subcontinent as all of these studies were done in the western population. Apart from the sigmoid colon and rectal mucosal biopsies, other anatomical sites have not been studied so far. Thus, there is much scope for research in this regard.

Several studies in Western countries have used Morphometry on large intestinal mucosal biopsies from patients with chronic Inflammatory Bowel Disease with variable results[54,96,97]. Jenkins et al, found that the distribution of cells in the mucosa, the height of epithelial cells and the difference in the length of the mucosa at the surface and at the base were features useful in discriminating Inflammatory Bowel Disorders[54]. Thompson et al[97] found significant difference in various morphological

parameters to differentiate between normal controls, acute self-limiting colitis and chronic IBD. One significant finding worth mentioning is that the height of rectal mucosa increased more in cases of UC (mean 650-750µm) than that of CD (500-550µm). Their strongest parameter in distinguishing UC and CD was the ratio of crypt epithelial cell height to luminal surface epithelial cell height. Zaitoun et al[96] only compared UC cases pre and post treatment and found similar results. These studies have used techniques and intricate methods like image analysers and special software to ensure accurate results which are very encouraging. However these are also not usually available to pathologists during routine reporting. In an earlier study from our institute, on intestinal tuberculosis and Crohn's disease, granuloma size in mucosal biopsies was measured using an eye-piece Graticule and was found to be significantly useful in distinguishing the two disorders[7]. Other inflammatory changes were not quantitated however in that study. To the best of our knowledge and despite extensive review of literature, we found no morphometric studies carried out on mucosal biopsies of patients with UC or CD in India.

In this present study we aim to evaluate a few simple morphometric parameters on mucosal biopsies from patients with and without inflammatory bowel disorders. The parameters chosen are based on the most important changes in the mucosa in Inflammatory Bowel Disease, namely, architectural alteration and chronic inflammation and will be simple enough to be used by both experienced and inexperienced pathologists. Our hypothesis is that some of these simple measures will

provide additional information useful in the diagnosis and differentiation of these disorders.

To the best of our knowledge, our study is going to be the first study in India to incorporate morphometric analysis of the mucosa to differentiate between CD, ITB and UC. Previous authors have worked on mucosal biopsies to differentiate between normal mucosa and UC and to differentiate between UC and CD only. Furthermore, most of these authors have only used rectal and sigmoid colonic mucosal biopsies. Our study will evaluate mucosal biopsies from the terminal ileum, caecum/ascending colon, transverse colon, descending colon, sigmoid colon and rectum.

The data based on normal biopsies that we generate will also be useful. For instance, the current diagnosis of eosinophilic colitis is based on the number of eosinophils in the intestinal mucosa of western individuals, which could be very different from that in our population.

AIM

To study a few simple morphometric features and conventional existing histological parameters in ileal and segmental colorectal mucosal biopsies from patients with Crohn's disease, intestinal Tuberculosis, Ulcerative Colitis and normal controls in order to identify features that will aid in the diagnosis and differentiation of these disorders.

OBJECTIVES

- To study and quantitate the histological features of CD, UC and ITB on terminal ileal and segmental colo-rectal mucosal biopsies.
- To study the mean heights of villi, crypts and mucosa in mucosal biopsies of controls, CD, UC and ITB and to evaluate whether they are useful in the differentiation of these disorders.
- To evaluate the mean number of plasma cells/ oil immersion field in the deep mucosa and eosinophils/ high power field in the normal Indian population.
- To evaluate the usefulness of eosinophils counts and plasma cell counts in the mucosa to differentiate between UC, CD and ITB.

MATERIALS AND METHODS

METHODS AND MATERIALS

Our study was performed in the Department of General Pathology, Christian Medical College, Vellore. Prior to the initiation of our study, we performed a pilot study. Our objective was to standardize methodology and to calculate sample size. It was decided to count plasma cells per oil immersion field (OIF) and eosinophils per high power field (HPF) in the deep part of the mucosa using an Olympus CH20i microscope. Based on the cell counts of our pilot study our statistician helped us in calculating the sample size and we decided to include 30 cases each of normal controls, Ulcerative colitis (UC), Crohn's disease (UC) and intestinal Tuberculosis (ITB).

Our study was approved by the Institutional Review Board and Ethics committee of Christian Medical College, Vellore.

All cases of UC, CD and ITB diagnosed between January 2008 and December 2013 were identified from our database in the Department of General Pathology. The final clinical diagnosis on each of these biopsies was confirmed based on relevant clinical history, the findings on clinical examination, laboratory parameters, radiological findings, treatment history and follow-up data that we collected from the Clinical workstation database In addition 30 cases with no significant lesion on histological examination normal mucosal study on endoscopy and no evidence of Ulcerative colitis, Crohn's disease or Tuberculosis on review of all clinical data were selected to serve as controls. 3 cases were positive for TB Gold Quantiferon, 4 cases were

positive for Xpert TB PCR, 6 cases grew M.tuberculosis on culture medium and in 11 cases acid fast bacilli was demonstrated on biopsy by special stains.

Inclusion Criteria:

- Only those cases were selected that had a histological diagnosis suggestive of the concerned disease (CD/UC/ITB) and a final clinical diagnosis of CD, UC or TB based on a combination of the clinical history, radiological studies, laboratory findings, endoscopy, histology, response to treatment and had at least 1 year of follow-up information.
- Normal mucosa controls were those cases in which both the colonoscopy and histological study were normal and there were no clinical features of IBD or TB.

Exclusion criteria:

- Cases in which the final histological or clinical diagnosis was ambiguous.
- If the slides were not available.
- Cases that did not have a minimum of 1 year follow up.
- Cases in which segmental biopsies from the ileum, caecum/ascending colon, transverse/descending colon and sigmoid colon/rectum were not available.
- If the entire biopsy was poorly oriented.
- Outside biopsies and outside slides sent to our institution for review were not included in our study since follow-up data was not available in those cases.

The first 30 cases of normal colonoscopic biopsies, CD, UC and ITB that fulfilled the above criteria were selected for the study.

Histological study

Haematoxylin and Eosin-stained sections of a total of 480 formalin-fixed, paraffin-embedded biopsies were studied. The slides were reviewed for a number of conventional histological parameters, initially by TV, and then by both TV and AP on a double headed research microscope without prior knowledge of the diagnosis. Blinding both the investigators to the diagnosis helped in eliminating 'investigator bias'. The following variables were evaluated and the findings recorded in a database created in Excel sheet

Severity of involvement of each segment: Those segments with moderate or severe involvement in terms of architectural alteration, inflammation and other findings were coded 2, while mildly involved segments were coded as 1. Uninvolved segments were coded 0.

Architectural alteration: The degree of change in the normal architecture was evaluated on low power examination and classified by a simple 3-tier system into mild, moderate and severe architectural alteration.

Chronic inflammation: The chronic inflammation in the mucosal biopsy was evaluated and classified subjectively by 3-tier system into mild, moderate and severe chronic inflammation.

Focal activity: The presence of focal infiltrates of neutrophils in the lamina propria or focal cryptitis was recorded.

Activity: The measure of disease activity based on neutrophil infiltration of the epithelium and lamina propria in each biopsy was classified as mild, moderate and severe. Any biopsy which had ulceration on microscopy was classified as having at least moderate activity

Crypt abscess: Each crypt abscess was counted and a total tally of each segment was recorded. We also classified segments as having<3 , 3-5 and >5 crypt abscess.

Granulomata: The presence or absence of necrotising and non-necrotising granulomas was recorded. The maximum diameter of each granuloma was measured using an eye-piece graticule (details given below). Total number of granulomas per biopsy site was also recorded.

Crypt branching and crypt disarray: The total number of crypts present in the biopsy specimen and also the number of crypts present in the diseased area of the mucosa were counted and recorded separately. The total number of branched crypts was recorded. Crypts displaying branching at the top (luminal portion) were not counted. The total number of crypts exhibiting disarray (axis not perpendicular to the

muscularis mucosa or significant alteration in crypt size in addition to crypt branching) were also counted.

The presence *of pyloric metaplasia, superficial erosion, deep ulceration, crypt atrophy, fibrosis and pseudo-villous change* were also recorded.

Morphometric analysis

Morphometric measurements were carried out using a simple commercially available Graticule. The Graticule has a scale bar 10mm in length, with each mm divided into 10 smaller parts. Each small division = 0.1mm = 100μm. The eyepiece graticule is located at the primary image of the microscope and is therefore in focus with the image of the specimen/ slide. It can be easily rotated for alignment and gives accurate measurements.

Areas in the biopsy that were well oriented and had the maximum inflammation and good tissue morphology free from artefacts were chosen for counting. Areas with ulceration and lymphoid follicles were always avoided.

Height of mucosa: Mucosal height was measured from the tip of the luminal surface epithelium till the upper-most portion of the muscularis mucosa. This parameter was measured using the 10X objective lens. 5 consecutive sites were measured and recorded. The numerical value obtained with the graticule was multiplied by 10 to arrive at the actual mucosal height in micrometers.

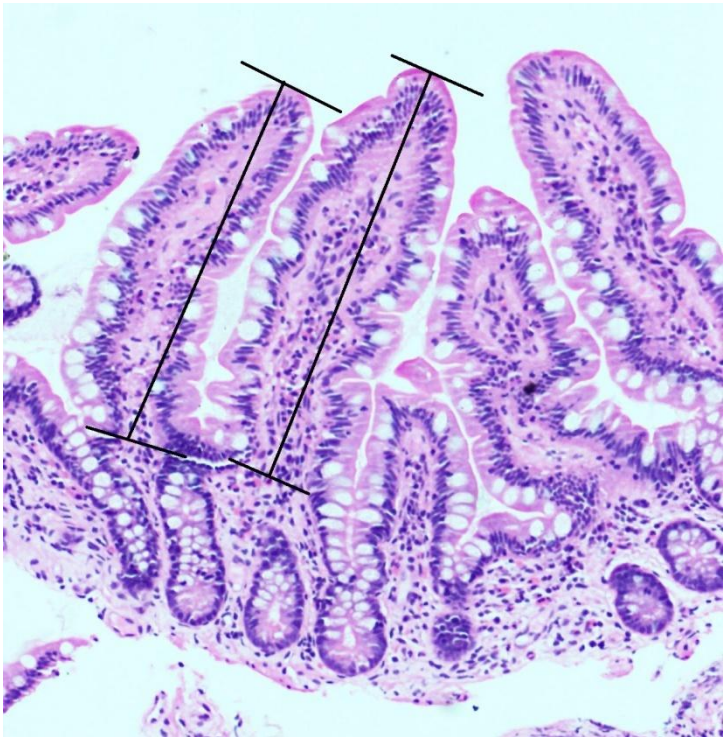
Height of crypts: The height of crypts was measured in areas with well oriented crypts. The measurement was taken from the tip of the crypt opening on the luminal surface till the bottom of the crypt. This parameter was measured using the 10X objective lens. 5 consecutive crypts were measured. The numerical value obtained with the graticule was multiplied by10 to arrive at the crypt height in micrometers.

Height of villi: The height of 5 villi was measured from the tip of the villous till the upper limit of the surface epithelium at the mouth of the adjacent crypt. This parameter was measured using the 10X objective lens. 5 well-oriented villi were measured and the numerical value obtained with the graticule was then multiplied by 10 to arrive at the villous height in micrometers.

Plasma cell quantification: Plasma cells were counted in the lower half of the mucosa just adjacent to the muscularis musosa under the oil-immersion field (100X lens). Plasma cells were defined as having an eccentrically placed nucleus with clumped , clock-faced chromatin, a peri-nuclear halo and amphophilic cytoplasm . Areas with maximum inflammation devoid of lymphoid follicles and ulceration were selected and counts were done in 5 consecutive fields. In segments that were not inflamed, the entire height of the mucosa fit into one oil-immersion field. In these cases the graticule was oriented horizontally, parallel to the muscularis mucosa in order to divide the mucosa into upper and lower halves. Plasma cells were then counted separately in the lower half (basal 34lasmacytosis) and the upper half of the field.

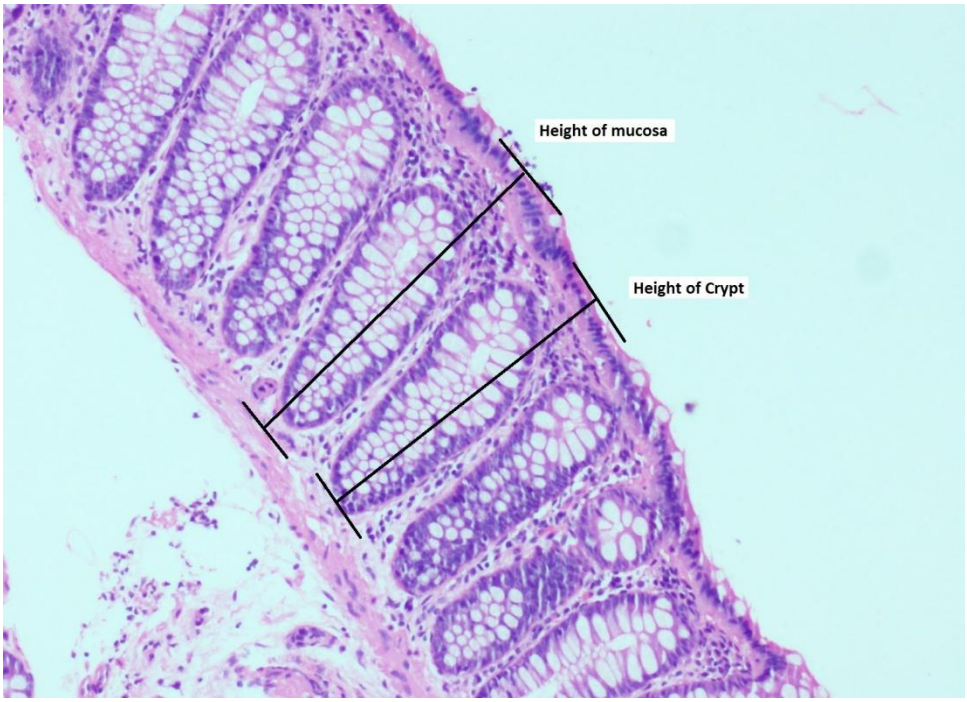
Eosinophil quantification: Using the high power lens (40X), lamina propria eosinophils were counted in 5 consecutive fields. Strict morphological criteria were used to exclude mast cells and neutrophils that may mimic eosinophilis. Cells with bi-lobed nuclei and orange granules in the cytoplasm were counted.

Granulomas: Maximum dimension of each granuloma was measured under the high-power field (40X). Total number of granulomas in each segment was also recorded. The numerical value obtained with the graticule was multiplied by 2.5 to arrive at granuloma size in micrometers.



Picture A: Height of 5 consecutive well oriented villi are measured from the base of the origin of a villous till its tip (100X magnification)

Picture B: Height of the mucosa is measured from the tip of 5 well oriented villi till the top most visible portion of the muscularis mucosa (100X magnification)



Picture C: Height of crypts and mucosa are measured in 5 consecutive fields with well oriented crypts free from Payer's patches and processing artefacts (100X magnification)

Statistical Analysis

Descriptive statistics for continuous variables were represented using mean and standard deviation. For categorical variables frequency and percentages are given. One-way analysis of variance (ANOVA) was used to the see the association between different groups and morphometric parameters (Heights of villi, crypts, mucosa and counts of plasma cells and eosinophils).

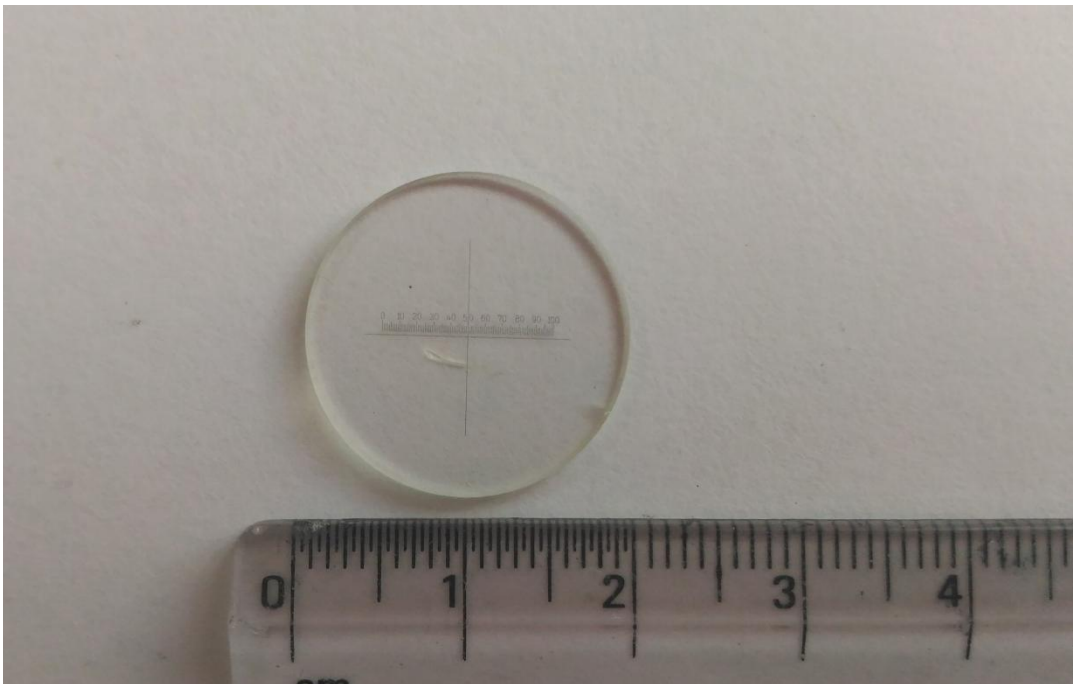
Bonferroni test was used to assess the post-HOC (intergroup correlation/ pair-wise analysis).

Association of histological parameters between the different groups was assessed using Fisher's exact test. Association of granuloma number and dimension with diseases was assessed using Kruskal-Wallis test. For comparing the normally distributed parameters between 2 groups independent sample t-test was used and for skewed distributed continuous variables Mann-Whitney U test was used.

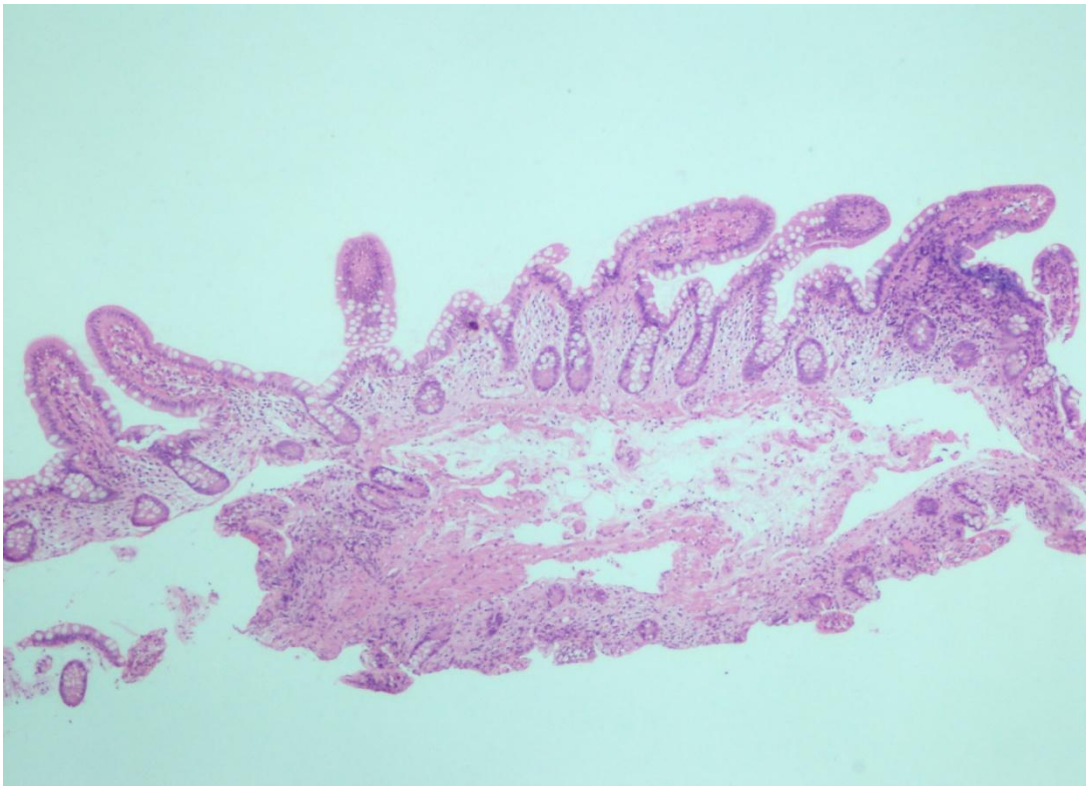
For calculating agreement between investigator 1 and investigator 2, ICC(Intra-class correlation coefficient was used) .

P-value of less than 0.05 was considered to be statistically significant for all the tests. Software STATA version 13.1 was used for the stastistical analysis.

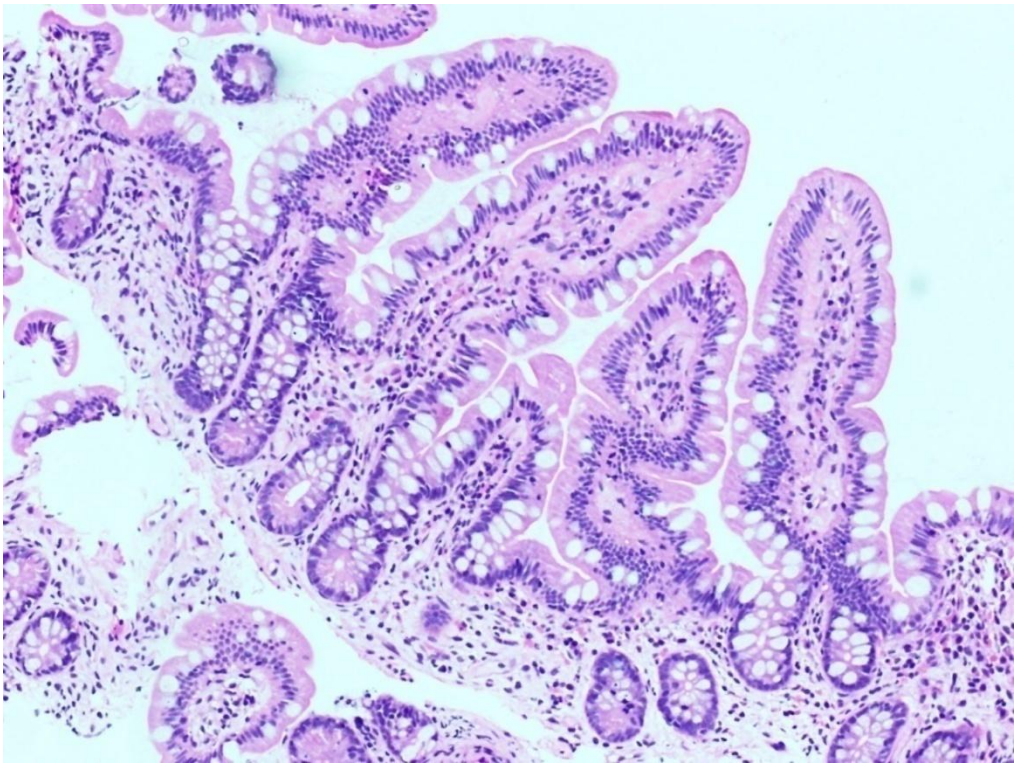
ILLUSTRATIONS



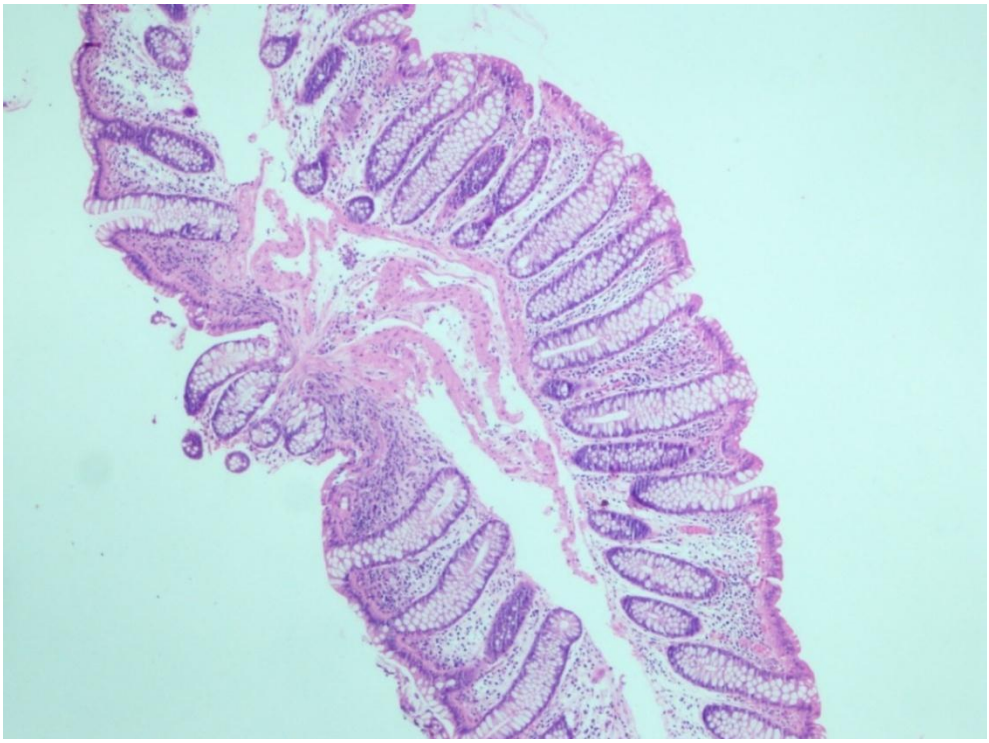
Picture 1: Eye-piece Graticule



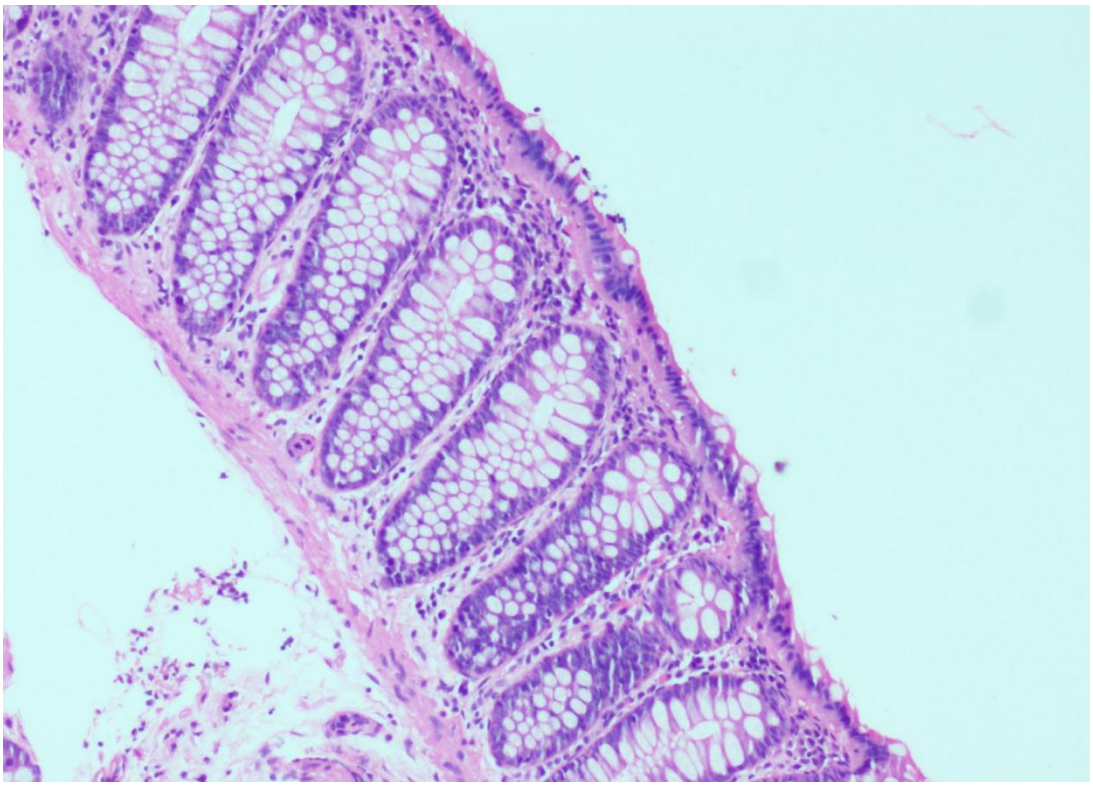
Picture 2: Normal ileal mucosal biopsy from a normal control (H&E, Magnification 40X)



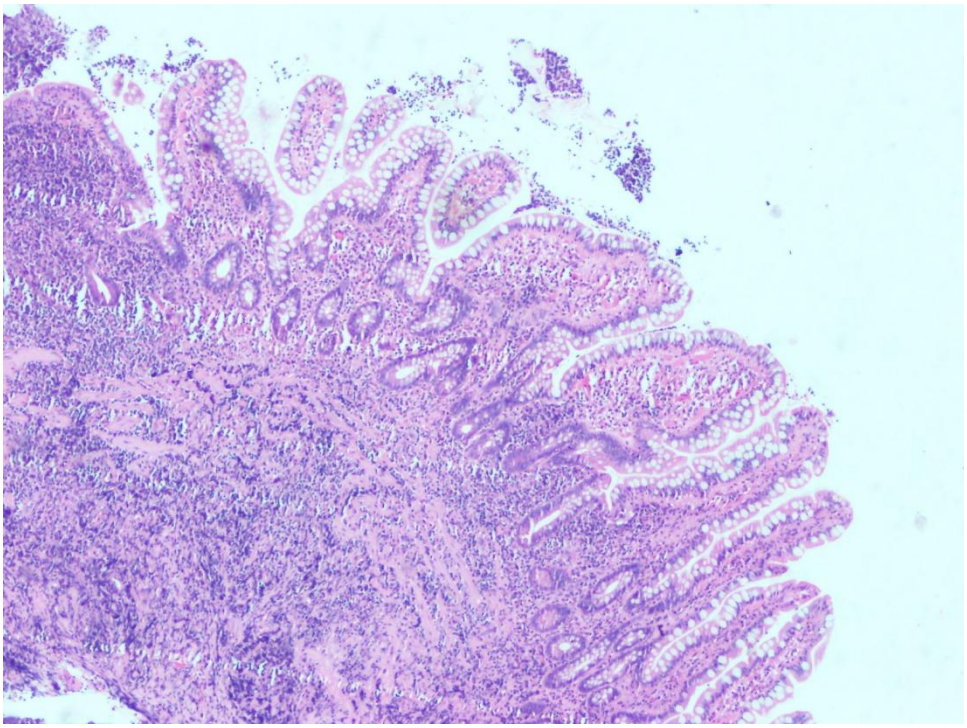
Picture 3: Normal ileum – mucosal biopsy from normal controls (H&E, Magnification 100X)



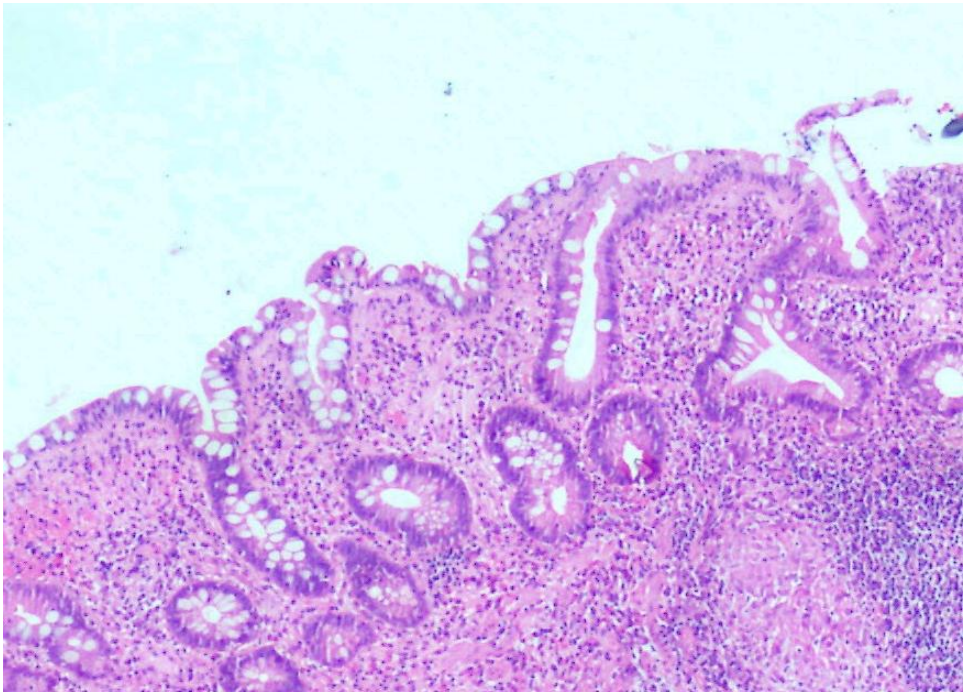
Picture 4: Normal colonic mucosal biopsy. Majority of crypts reach the muscularis mucosae and are arranged in parallel, described as a "test-tube" arrangement (H&E, Magnification 40X)



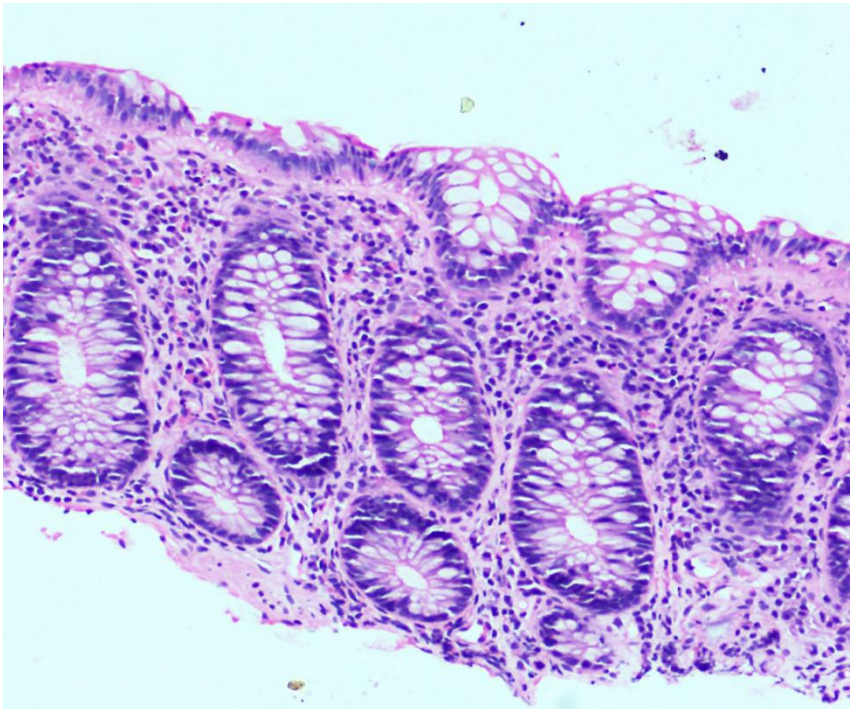
Picture 5: Normal colonic mucosal biopsy. Plasma cell gradient is maintained with majority of inflammatory infiltrate concentrated towards the luminal half (H&E, Magnification 100X)



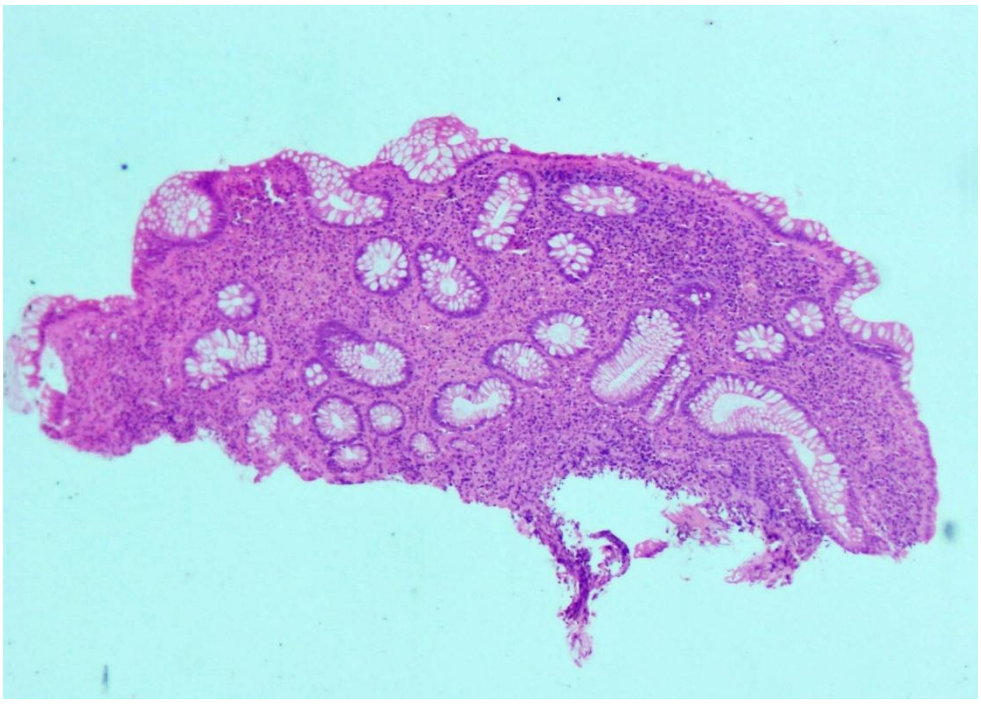
Picture 6: Mild broadening and blunting of villi with mild lamina propria inflammation (H&E, Magnification 400X)



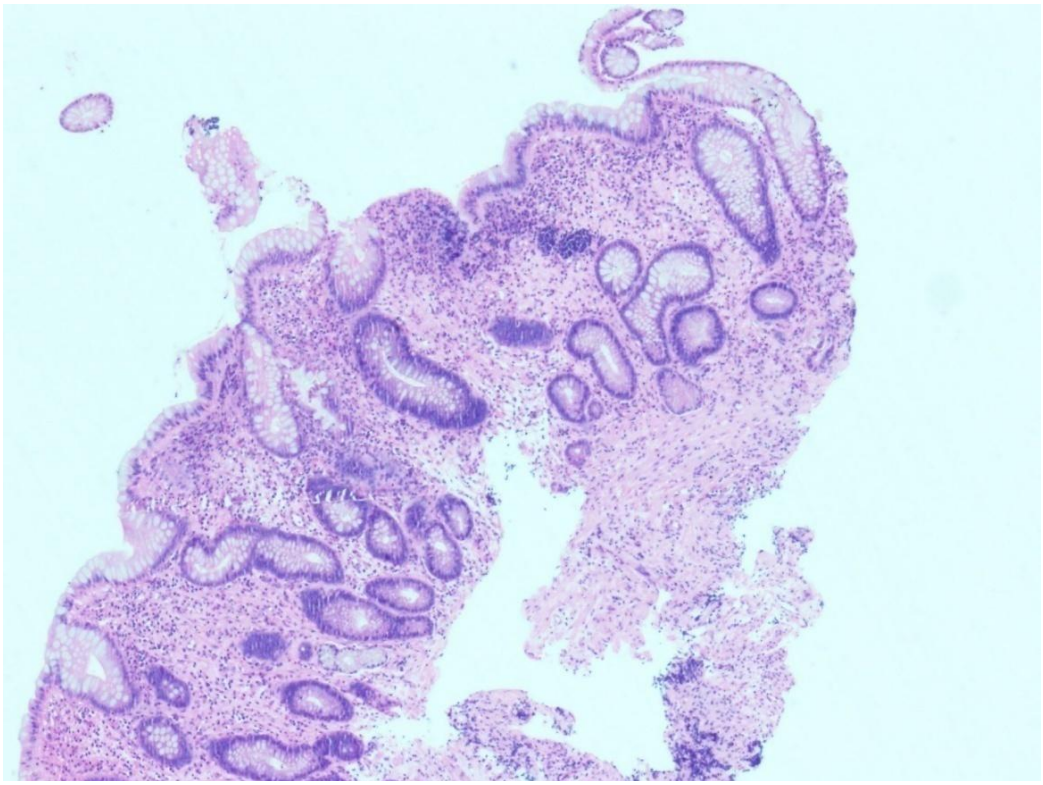
Picture 7: Moderate villous blunting in a case of ileal Tuberculosis with granulomatous inflammation (bottom right), (H&E, Magnification 100X)



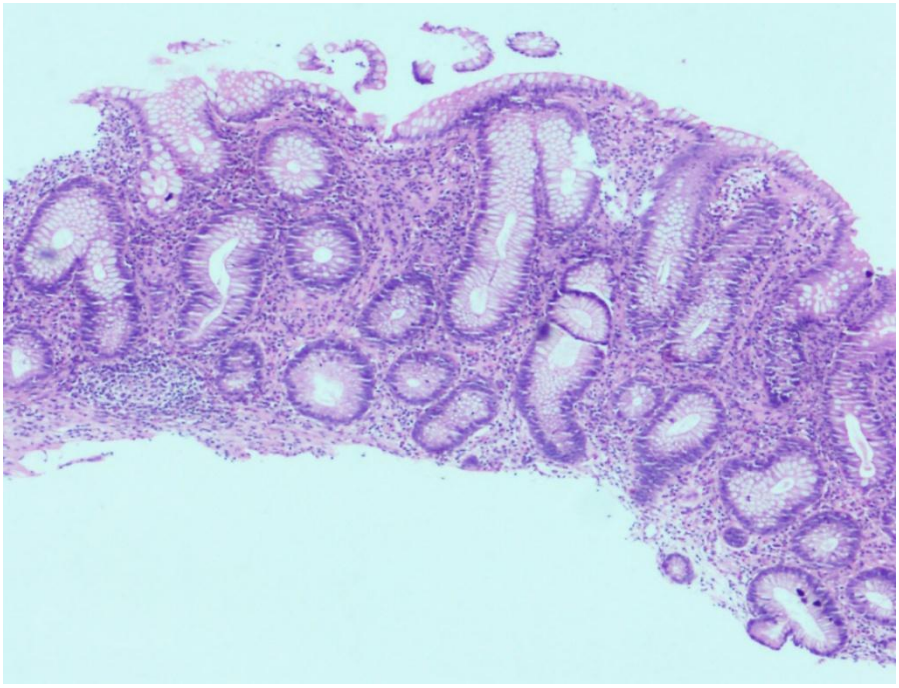
Picture 8: Colonic mucosal biopsy with mild chronic inflammation. This photomicrograph illustrates the beginning of loss of normal plasma cell gradient in lamina propria (H&E, Magnification 100X)



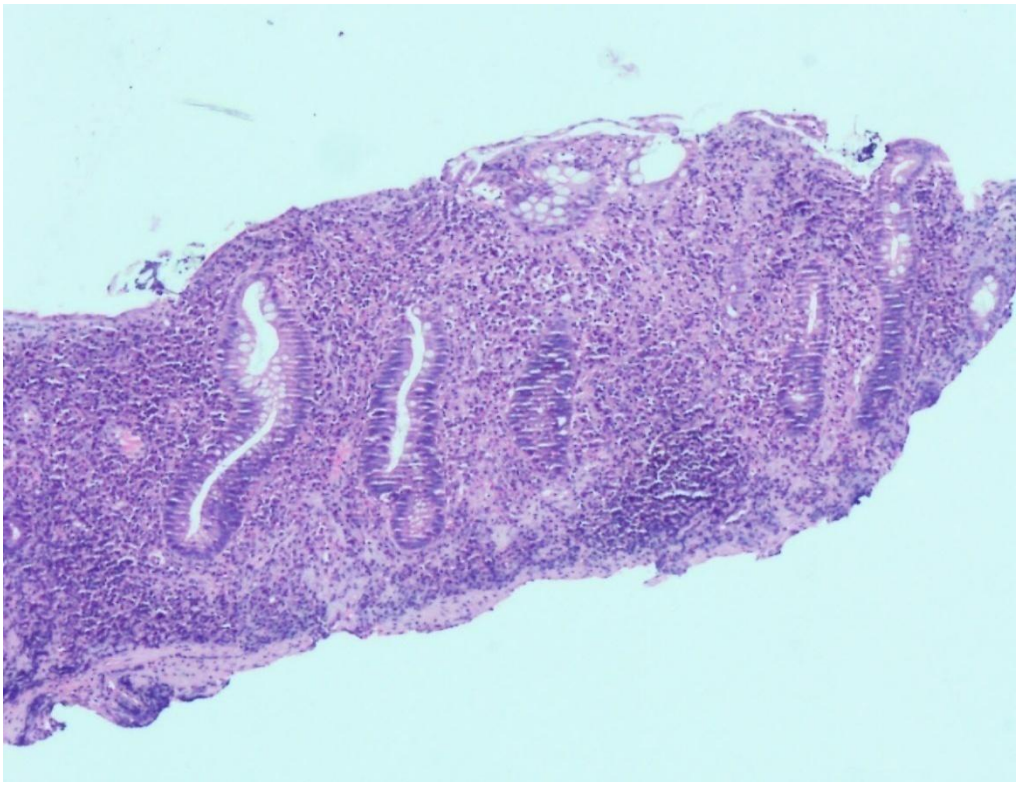
Picture 9: Colonic mucosal biopsy with severe chronic inflammation. This photomicrograph also illustrates moderate crypt disarray (H&E, Magnification 40X)



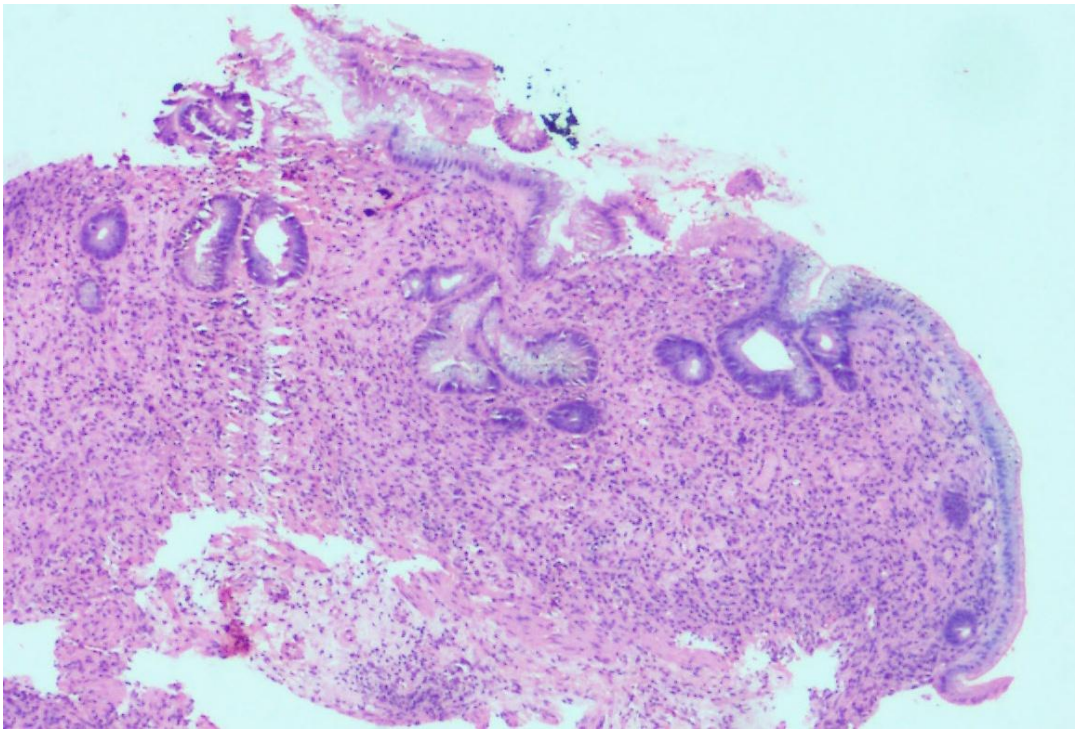
Picture 10: Mild crypt disarray in a colonic biopsy (H&E, Magnification 40X)



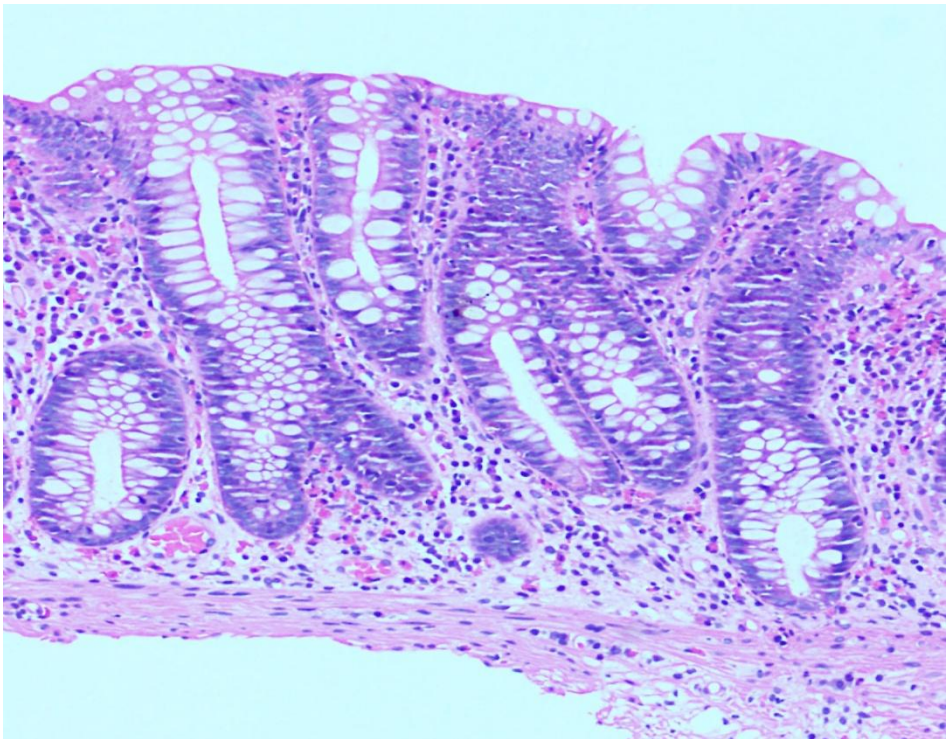
Picture 11: Moderate chronic inflammation and loss of plasma cell gradient in colonic biopsy (H&E, Magnification 100X)



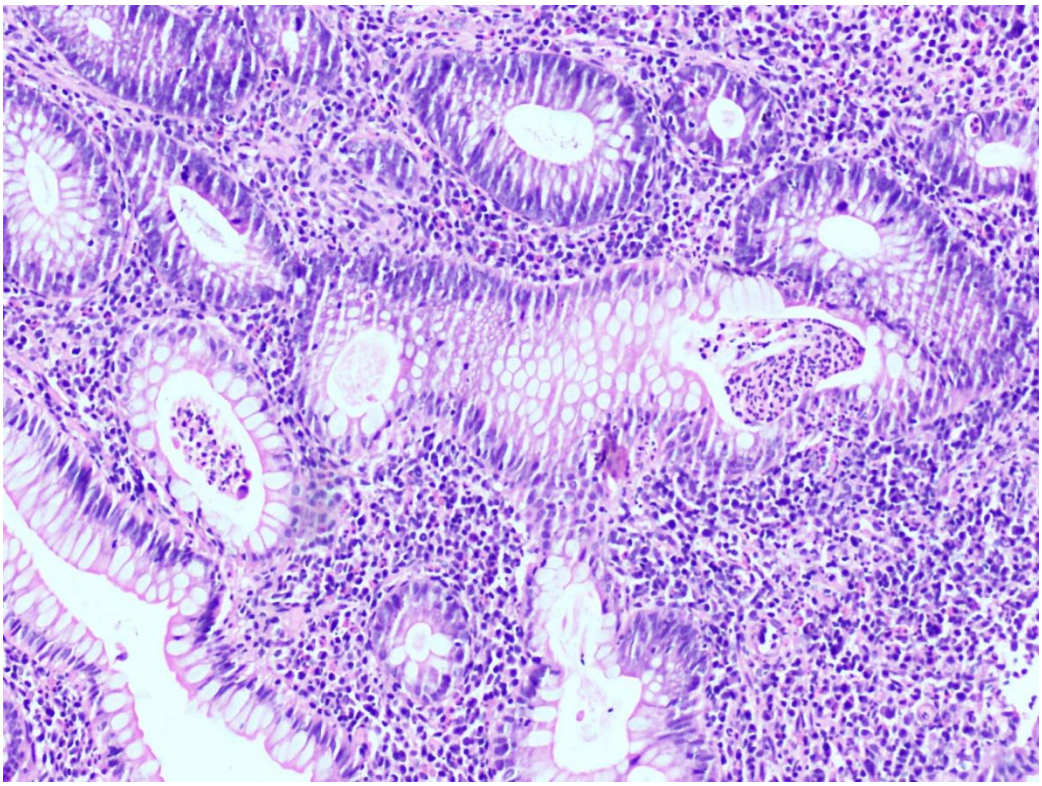
Picture 12: Severe chronic inflammation in a colonic biopsy (H&E, Magnification 50X)



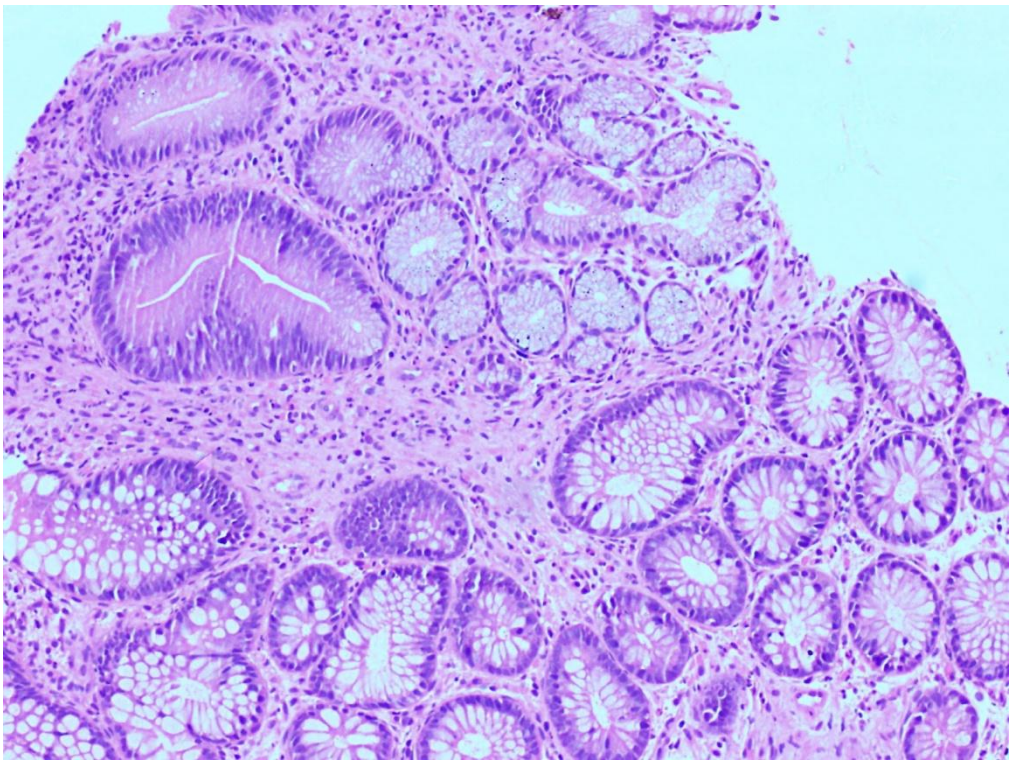
Picture 13: Severe architectural alteration with moderate to severe crypt atrophy and crypt loss, colorectal biopsy(H&E, Magnification 40X)



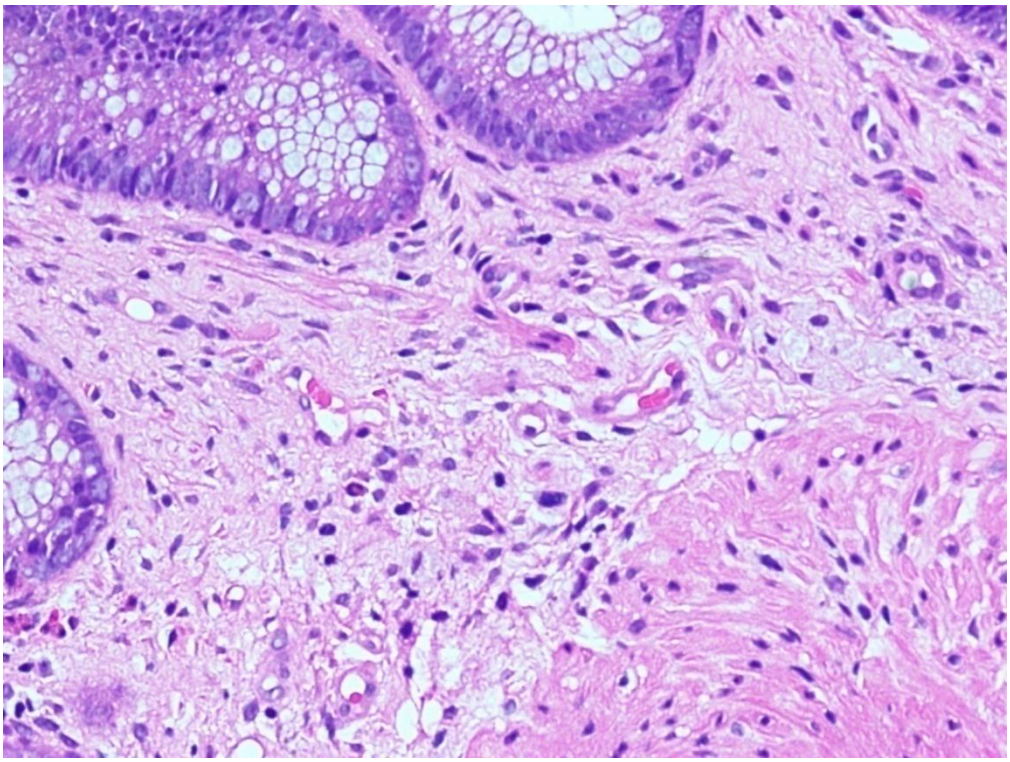
Picture 14: Crypt branching. Presence of is considered significant only in the lower part of the mucosa, not the luminal surface (H&E, 100X magnification).



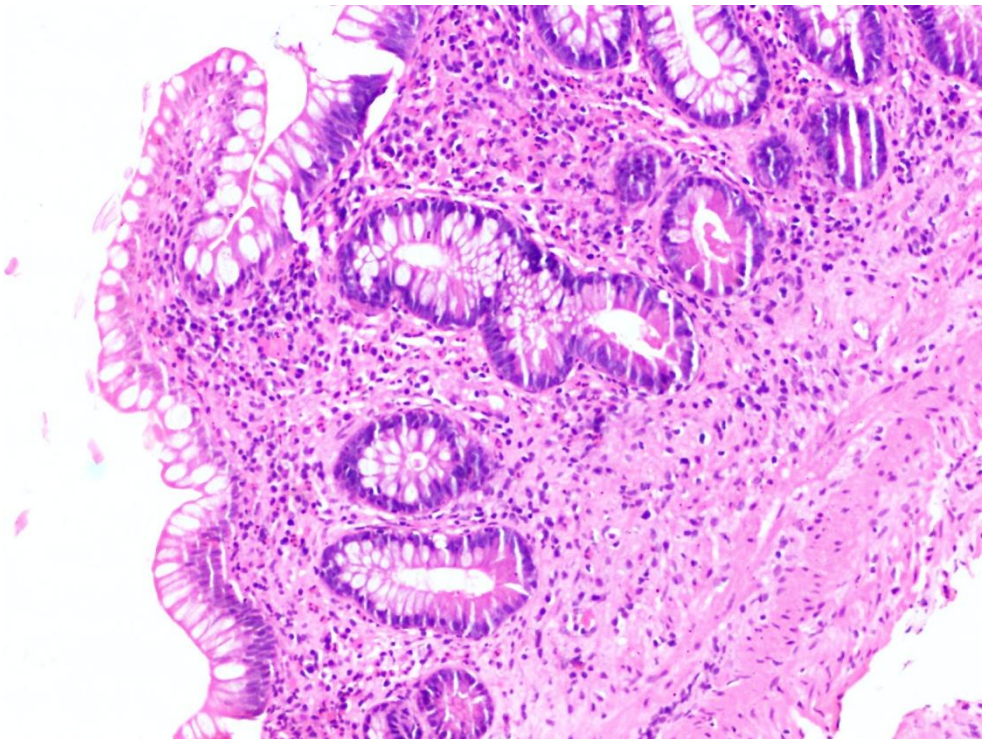
Picture 15: Photomicrograph demonstrating cryptitis and crypt abscesses (100X magnification)



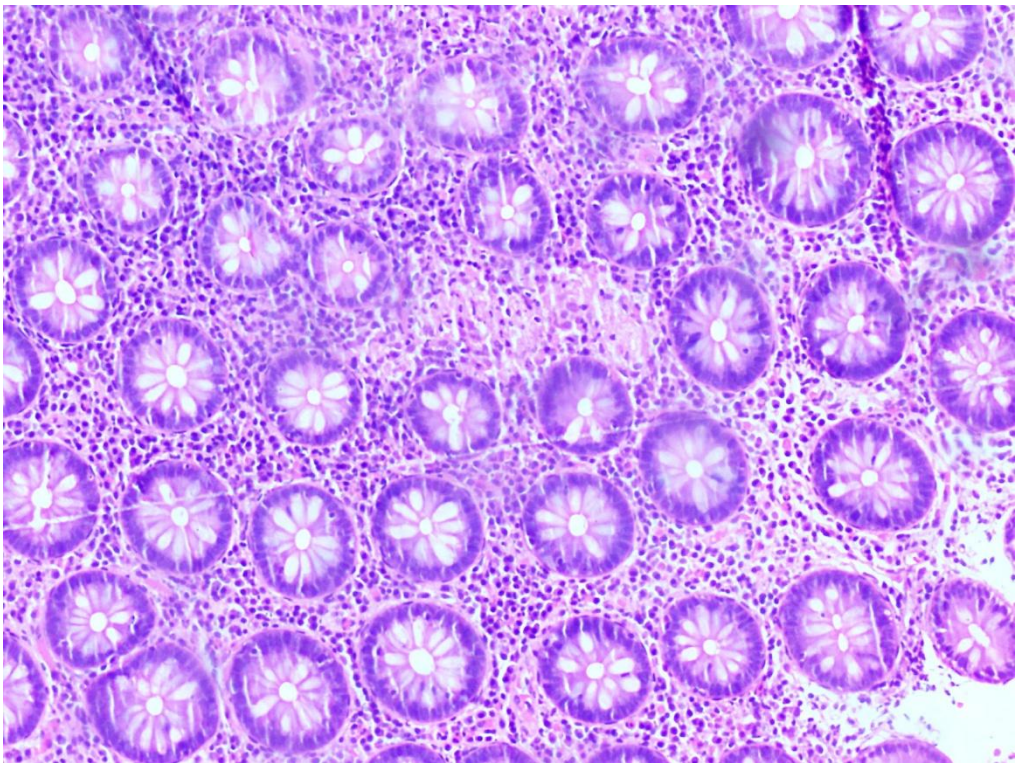
Picture 16: Pyloric gland metaplasia in caecum/ascending colonic mucosal biopsy (H&E, 100X magnification)



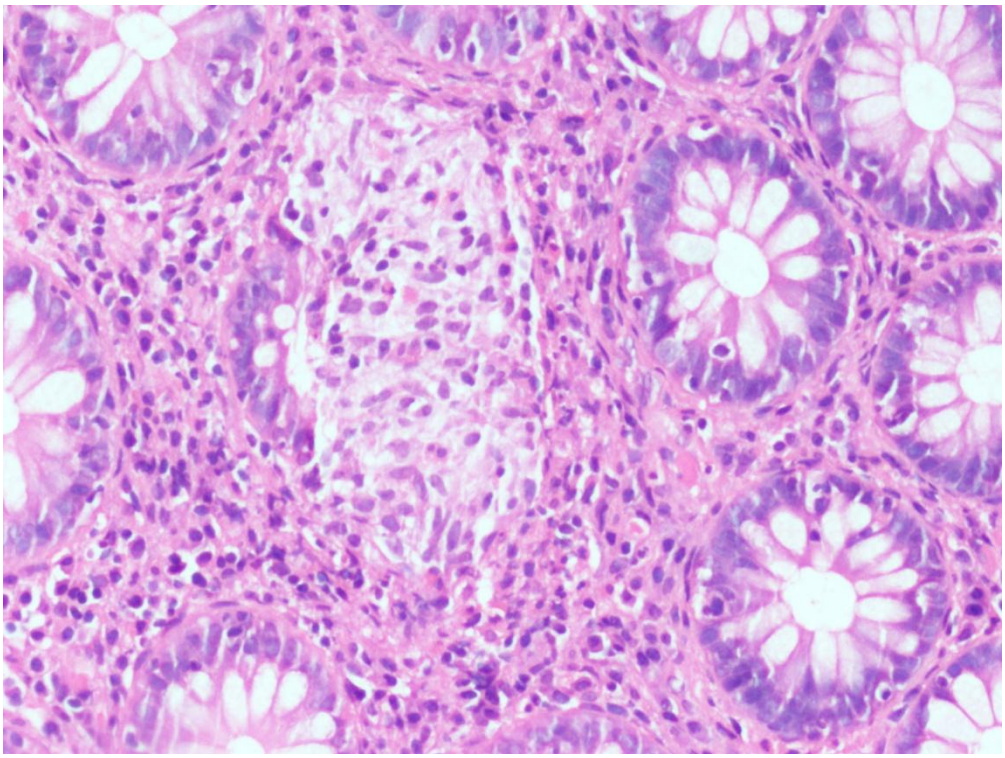
Picture 17: Fibrosis in a colonic mucosal biopsy (H&E, magnification 400X)



Picture 18: Fibrosis in an ileal mucosal biopsy (H&E, magnification 100X)

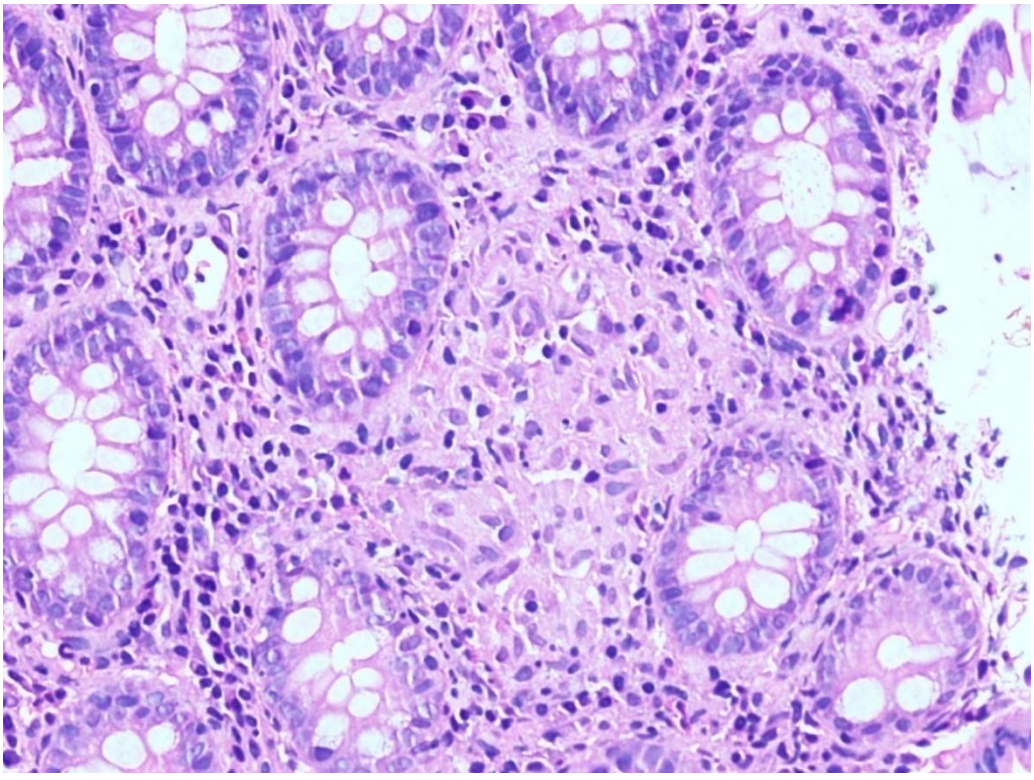


Picture 19: Case of Crohn's disease displaying a small granuloma (H&E, 100X magnification)



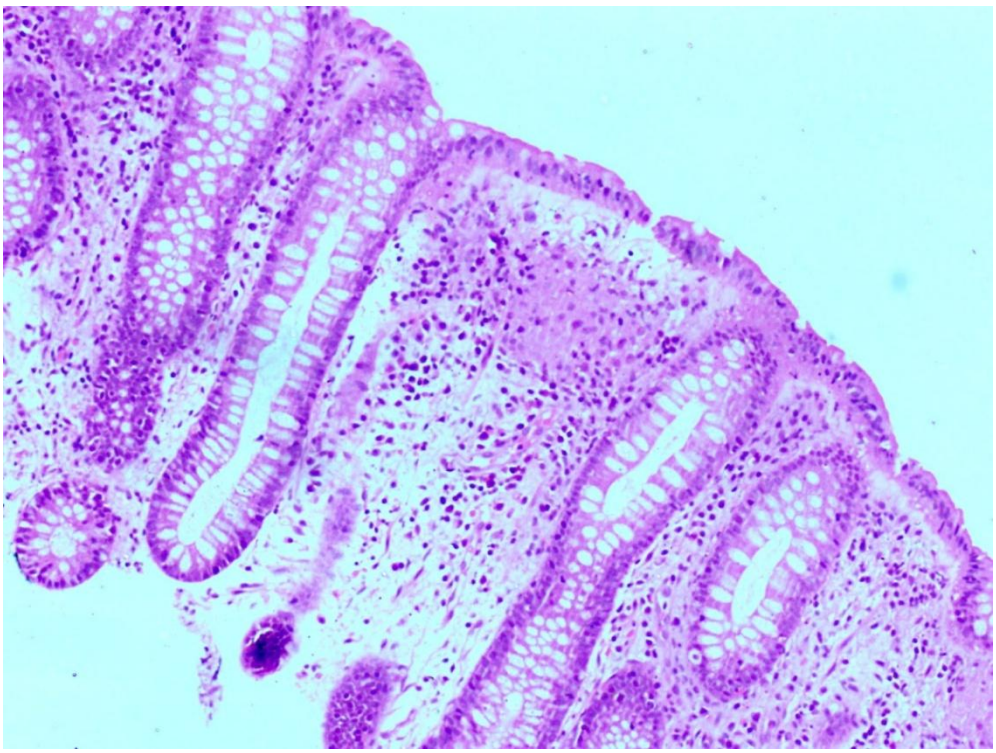
Picture 20: Peri-cryptal granuloma from a case of Crohn's disease (H&E, 400X magnification)

Picture 21: Case of Crohn disease displaying mild chronic inflammation, no significant architectural alteration and a small granuloma (arrow) (H&E, 40X magnification)

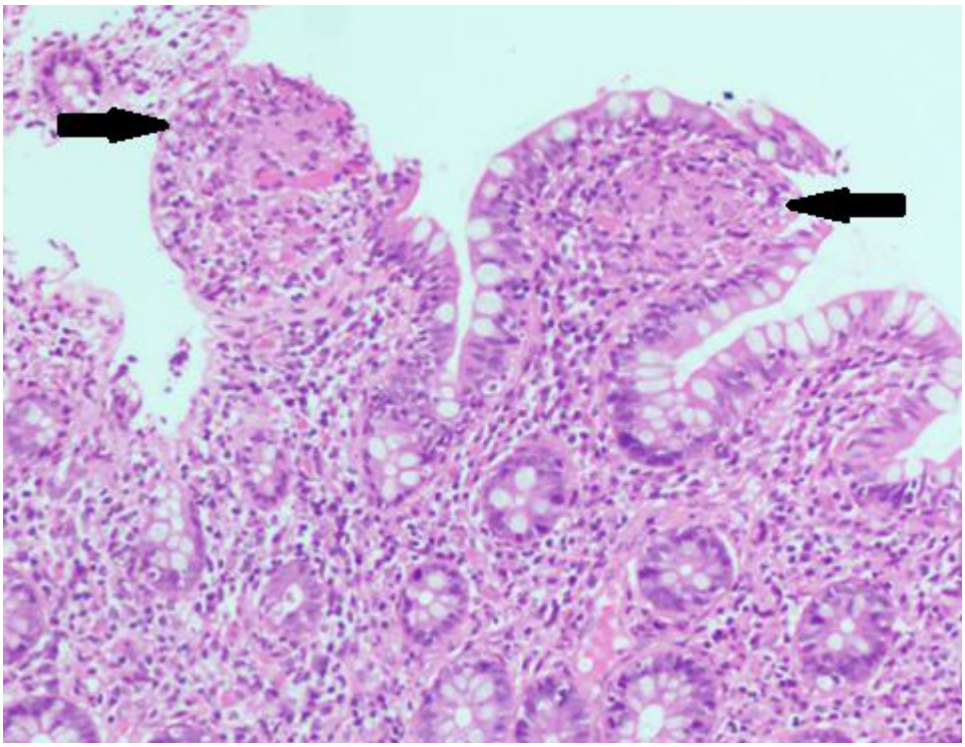


Picture 22: Same case of Crohn's disease (shown above) exhibiting small granuloma (H&E, 400X magnification)

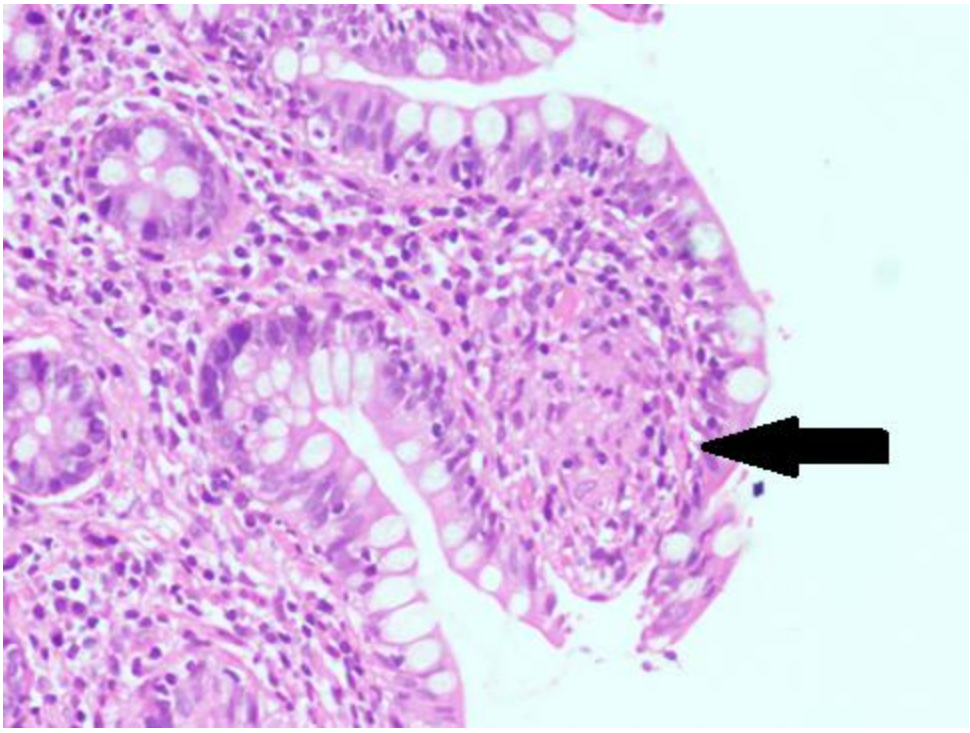
Picture 23: Case of Crohn's disease with 2 small granulomas (arrows) and no significant inflammation (H&E, 40X magnification)



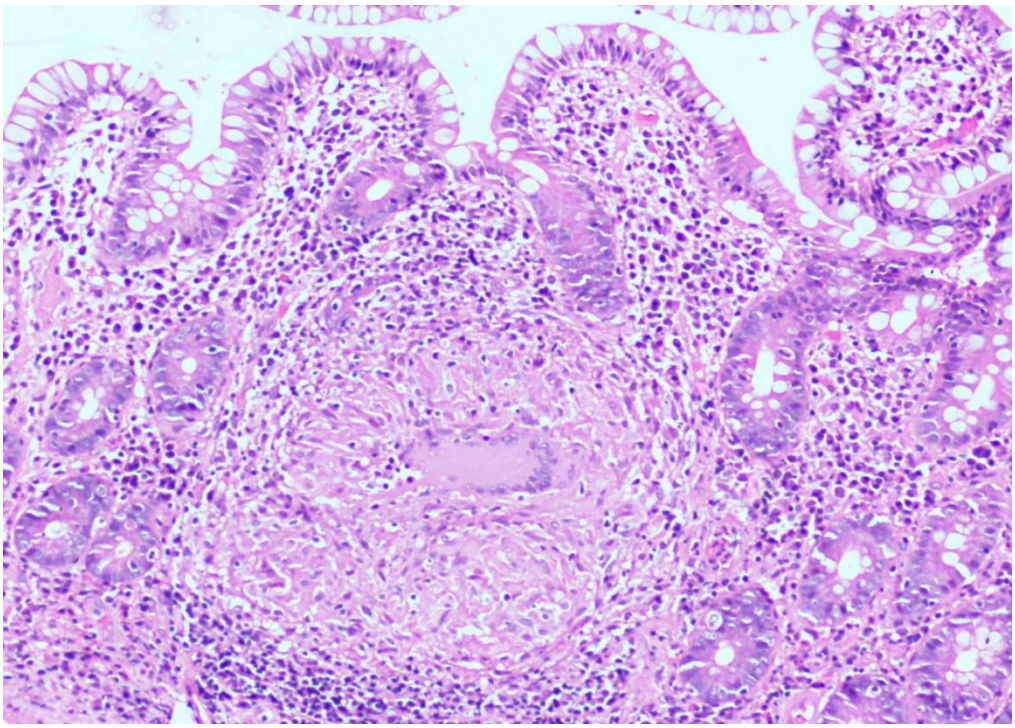
Picture 24: Same case of Crohn's disease (shown above) illustrating a small well-formed granuloma and minimal chronic inflammation within the lamina propria. (100X magnification)



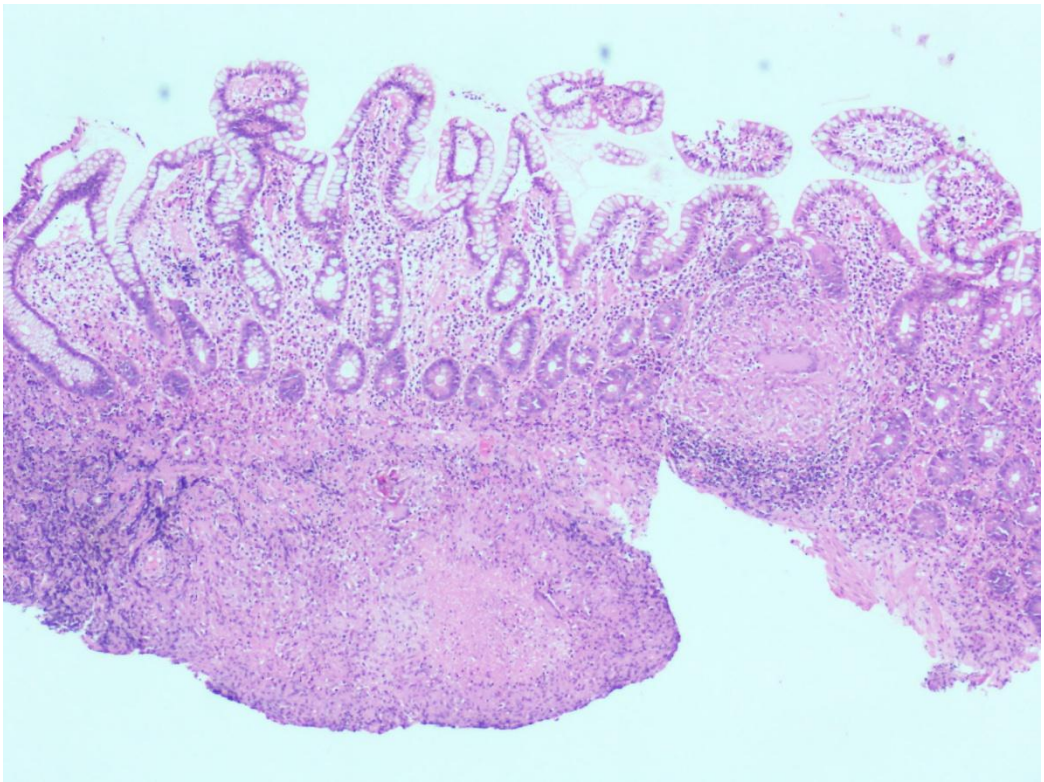
Picture 25: Case of Crohn disease with 2 small granulomas in the villi accompanied with mild chronic inflammation (H&E, 100X magnification)



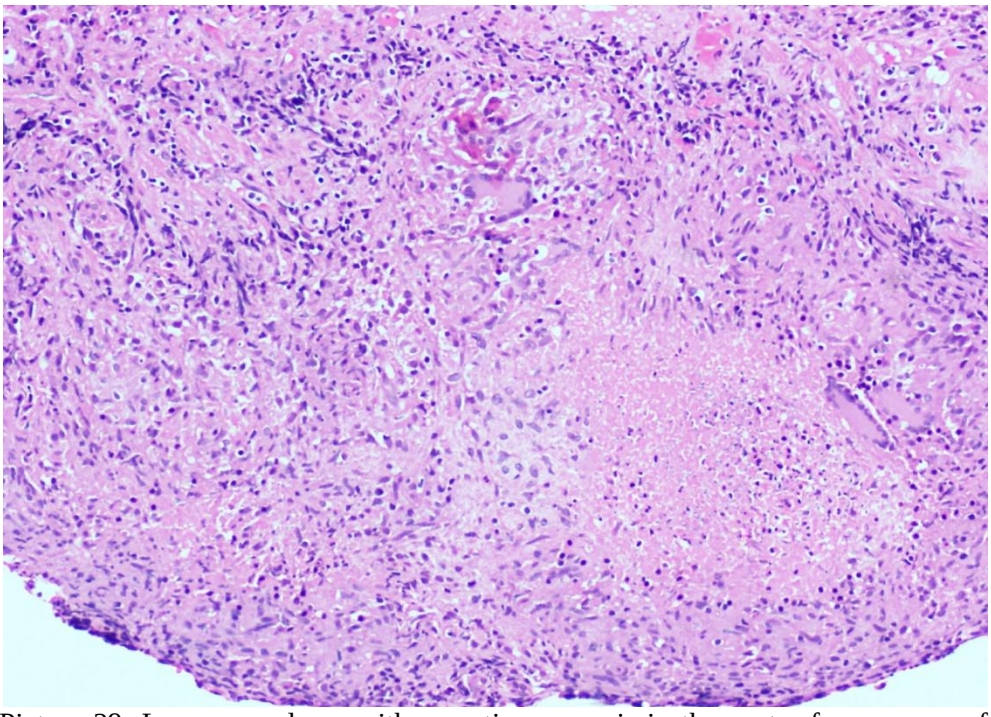
Picture 26: High power view of the above case illustrating small granuloma of Crohn disease (H&E, 400X magnification)



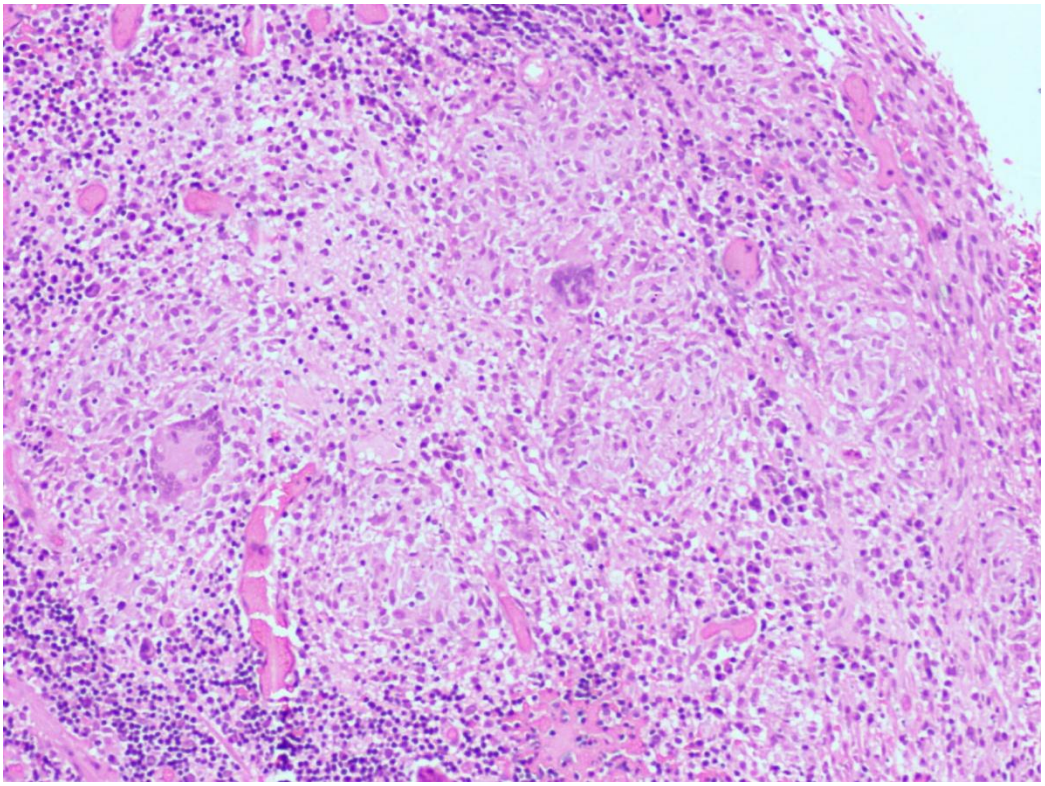
Picture 27: Medium sized granuloma (200 – 400 micrometers in dimension) from a case of Ileal Tuberculosis (H&E, 100X magnification)



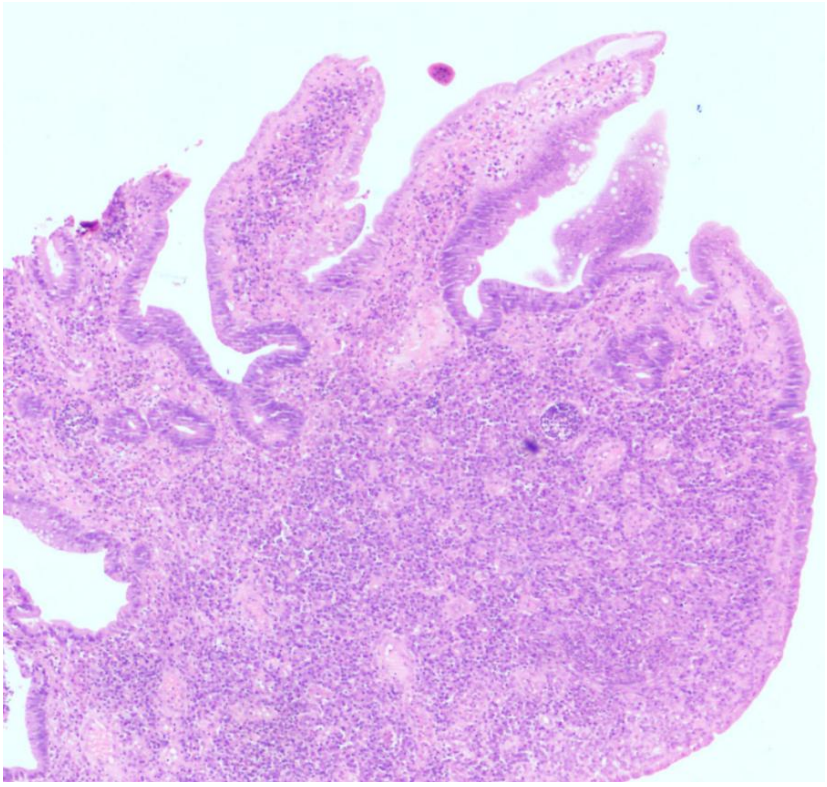
Picture 28 : A medium sized and a large granuloma (> 400 micrometers) with central necrosis from a case of ileal Tuberculosis (H&E, 40X magnification)



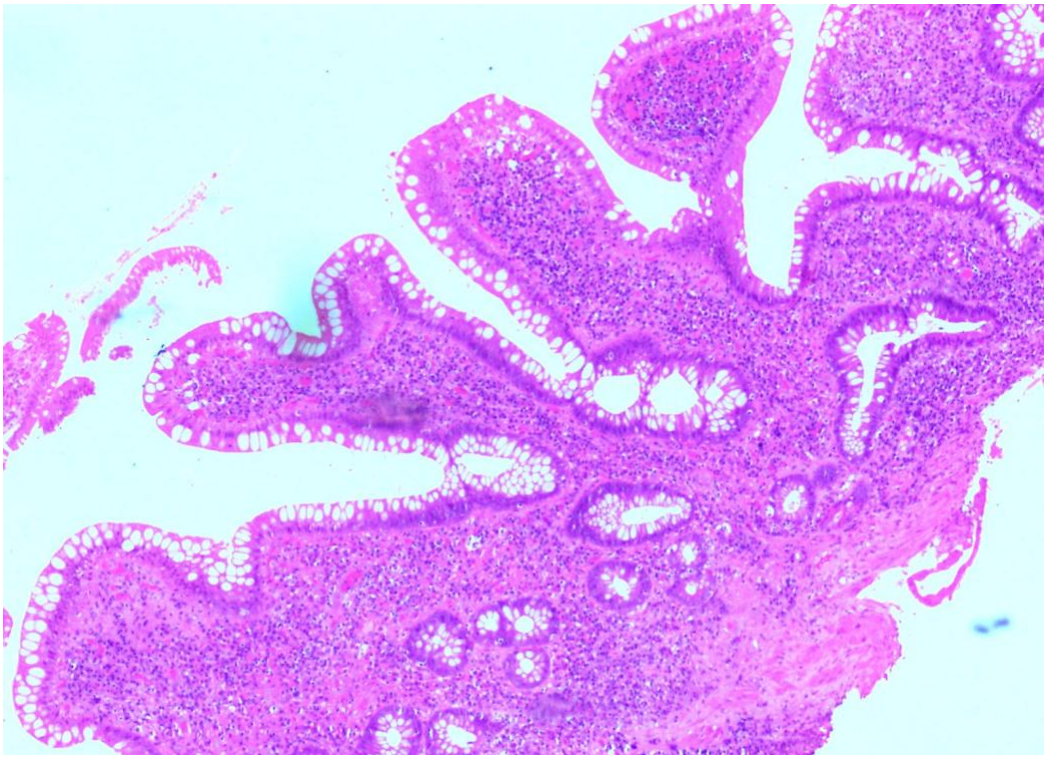
Picture 29: Large granuloma with caseating necrosis in the centre from a case of intestinal Tuberculosis (H&E, 100X magnification)



Picture 30: Confluent large granulomas from a case of caecal tuberculosis (H&E, 100X magnification)



Picture 31: Pseudo-villous change from a case of ulcerative colitis, rectal biopsy (H&E, 40X magnification)



Picture 32: Pseudo-villous change in a case of Ulcerative colitis, sigmoid/rectal biopsy (H&E, 40X magnification)

TABLES AND CHARTS

ILEUM

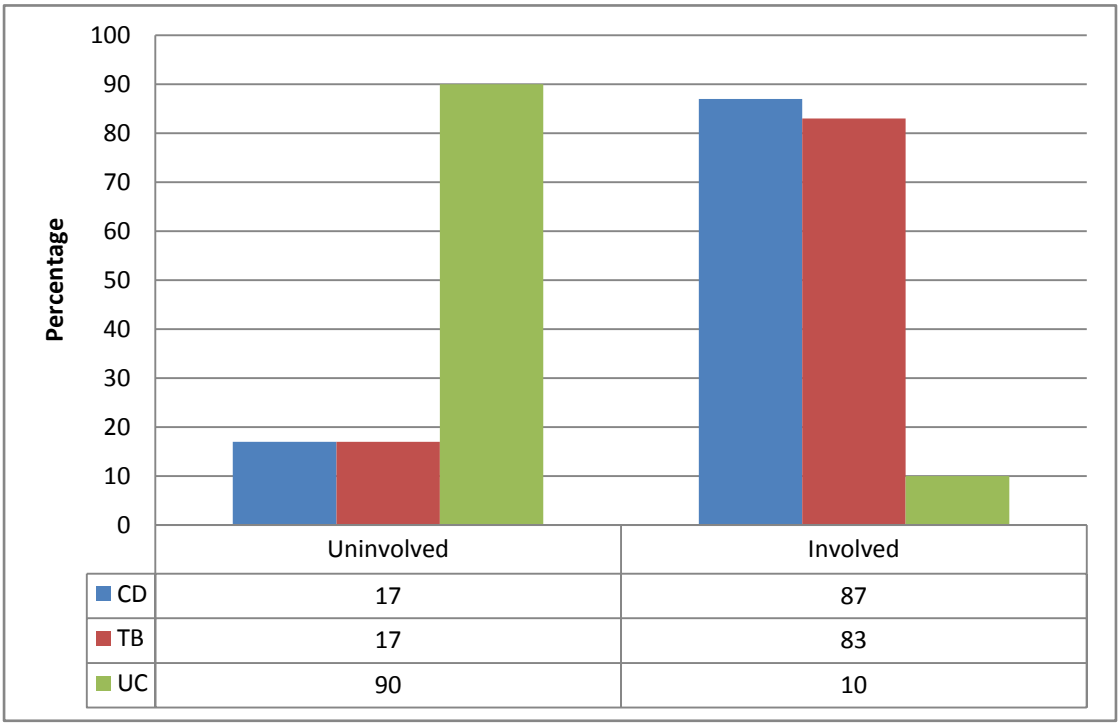


Figure 1: Proportion of ileal mucosal biopsies involved in disease

Table 1: Proportion of cases showing chronic inflammation in ileum.

	Controls	CD	ITB	UC
None	28 (93.3%)	4 (13.3%)	0	17 (56.7%)
Mild	2 (6.7%)	10 (33.3%)	6 (20%)	9 (30%)
Moderate	0	15 (50%)	22 (73.3%)	4 (13.3%)
Severe	0	1 (3.3%)	2 (6.7%)	0

Table 2: Proportion of cases showing architectural alteration in ileum.

	Controls	CD	ITB	UC
None	30	10 (33.3%)	5 (16.7%)	25 (83.3%)
Mild	0	12 (40%)	17 (56.7%)	3 (10%)
Moderate	0	8 (26.7%)	7 (23.3%)	2 (6.7%)
Severe	0	0	1 (3.3%)	0

Table 3: Proportion of focal activity, pyloric gland metaplasia, ulcers, fibrosis, pseudovillous change and atrophy in ileum.

	Controls	CD	ITB	UC
Focal activity	0	1 (3.3%)	2 (6.7%)	1 (3.3%)
Pyloric gland metaplasia	0	5 (16.7%)	8 (26.7)	1 (3.3)
Deep Ulcers	0	9 (30%)	20 (66.7%)	0
Fibrosis	4 (13.3%)	8 (26.7%)	16 (53.3%)	7 (23.3%)
Pseudovillous change	0	0	0	0
Atrophy	0	0	0	0

Table 4: Proportion of cases showing activity in ileum

	Controls	CD	ITB	UC
Absent	30	8 (26.7)	4 (13.3)	25 (83.3)
Mild	0	13 (43.3)	7 (23.3)	5 (16.7)
Moderate	0	9 (30)	19 (63.3)	0

Table 5: Proportion of cases showing cryptitis in ileum

	Controls	CD	ITB	UC
Absent	30	19 (63.3)	11 (36.7)	28 (93.3)
Mild	0	10 (33.3)	17 (56.7)	2 (6.7)
Moderate	0	1 (3.3)	2 (6.7)	0

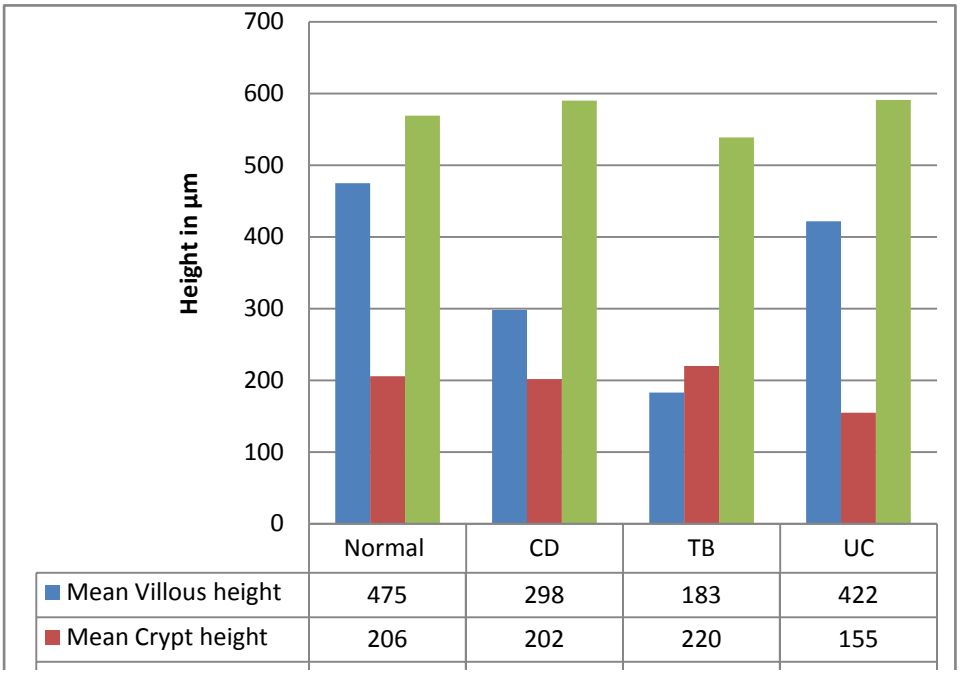


Figure 2: Morphometric measurements of ileal mucosa in diseases and controls. (Expressed in µm)

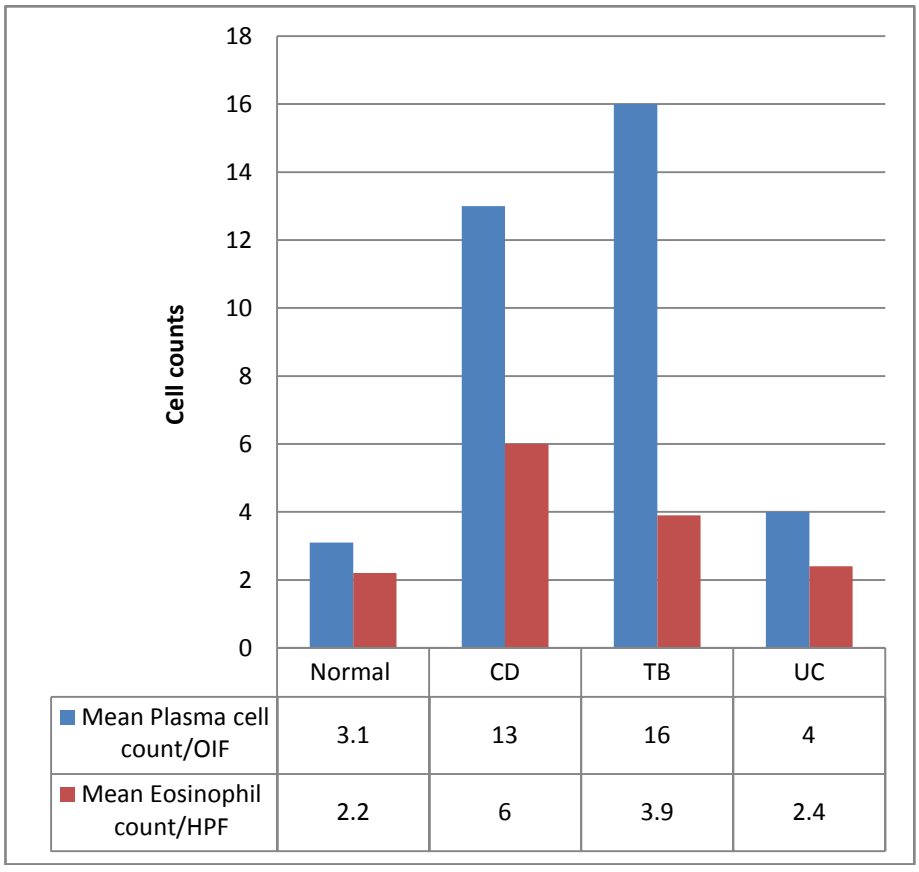


Figure 3 : Mean Cell counts in the ileum (Plasma cells/OIF; Eosinophils/HPF)

CAECUM/ ASCENDING COLON

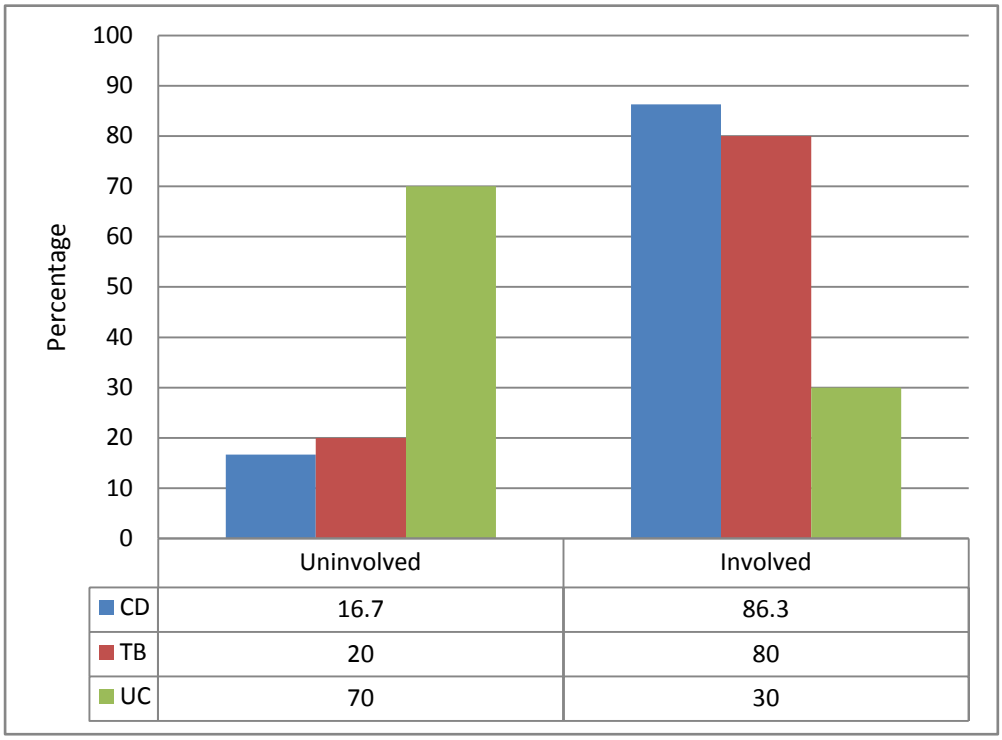


Figure 4: Proportion of caecum/ascending colon biopsies involved in disease

Table 6: Proportion of cases showing architectural alteration in caecum/ascending colon

	Controls	CD	ITB	UC
None	30	14 (46.7%)	9 (30%)	22 (73.3%)
Mild	0	11 (36.7%)	16 (53.3%)	7 (23.3%)
Moderate	0	5 (16.7%)	5 (16.7%)	1 (3.3%)

Table 7: Proportion of cases showing chronic inflammation in caecum/ascending colon.

	Controls	CD	ITB	UC
None	21 (70%)	5 (16.7%)	1 (3.3%)	6 (20%)
Mild	9 (30%)	11 (36.7%)	8 (26.7%)	21 (70%)
Moderate	0	10 (33.3%)	14 (46.7%)	2 (6.7%)
Severe	0	4 (13%)	7 (23.3%)	1 (3.3%)

Table 8: Proportion of deep ulcers, fibrosis, pyloric gland metaplasia and pseudovillous change in caecum/ascending colon.

	Controls	CD	ITB	UC
Deep Ulcers	0	4 (13.3%)	20 (66.7%)	1 (3.3%)
Fibrosis	0	5 (16.7%)	18 (60%)	3 (10%)
Pyloric gland metaplasia	0	2 (6.67%)	0	0
Pseudovillous change	0	0	0	1 (3.3%)

Table 9: Proportion of cases showing activity in caecum/ascending colon.

	Controls	CD	ITB	UC
None	30	9 (30%)	4 (13.3%)	14 (46.7%)
Mild	0	14 (46.7%)	7 (23.3%0	13 (43.3%)
Moderate	0	7 (23.3%)	18 (60%)	3 (10%)
Severe	0	0	1 (3.3%)	0

Table 10: Proportion of cases showing cryptitis in caecum/ascending colon

	Controls	CD	ITB	UC
None	30	13 (43.3%)	8 (26.7%)	15 (50%)
Mild	0	13 (43.3%)	19 (63.3%)	15 (50%)
Moderate	0	4 (13.3%)	3 (10%)	0

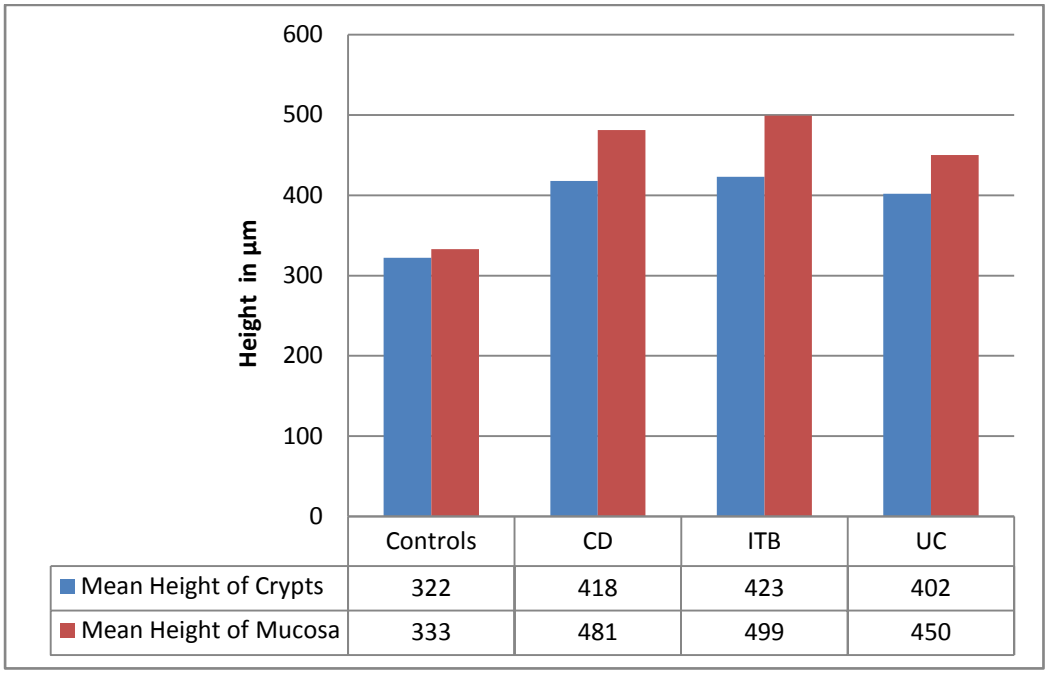


Figure 5: Morphometric measurements of caecum/ ascending colonic mucosa in diseases and controls (Expressed in µm)

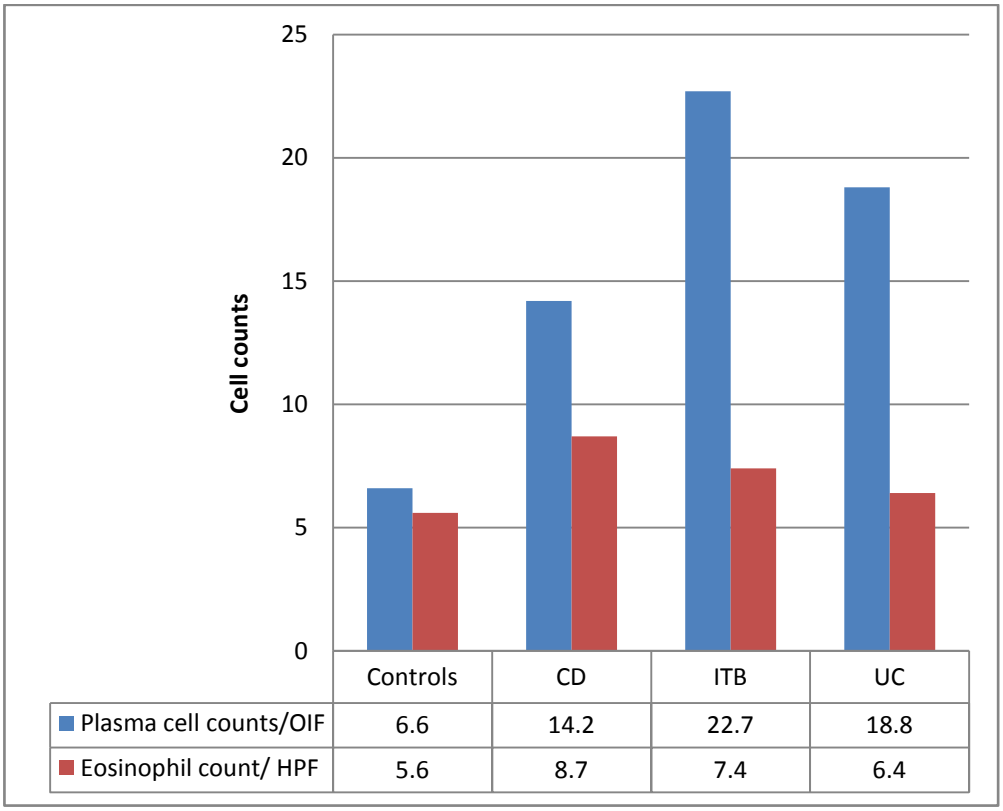


Figure 6: Mean cell counts in the caecum/ascending colon (Plasma cells/OIF, Eosinophils/HPF)

TRANSVERSE/ DESCENDING COLON

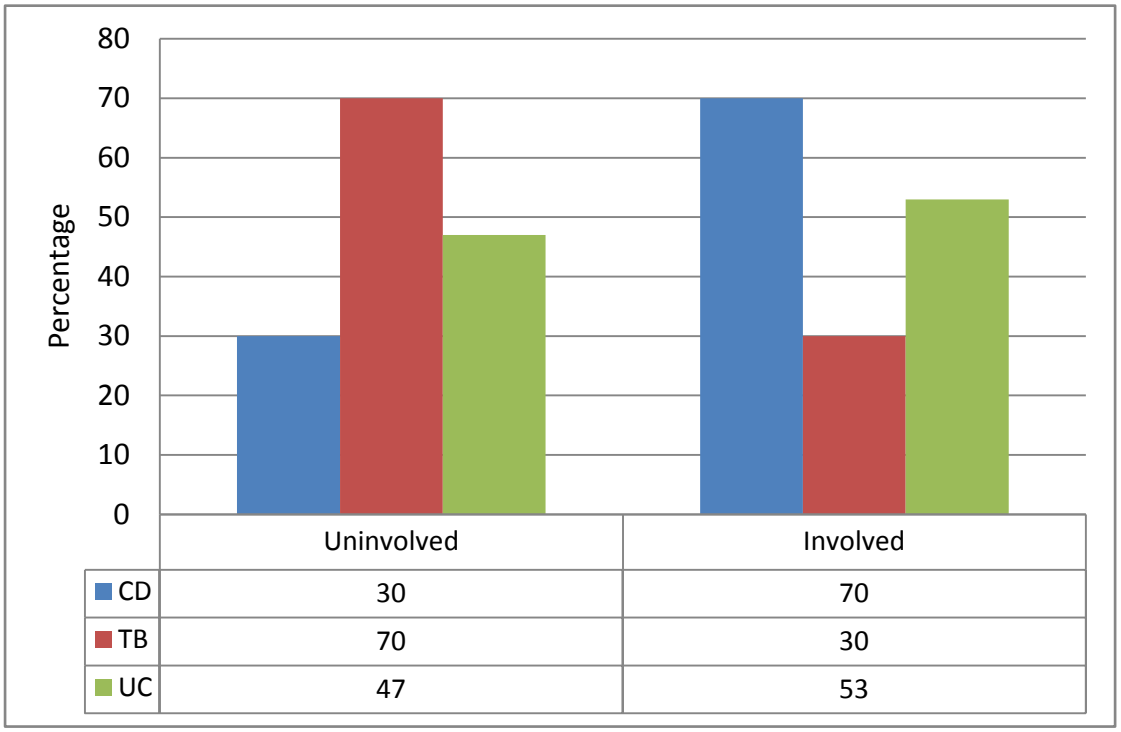


Figure 7: Proportion of transverse/descending colon biopsies involved in disease

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Table 11: Proportion of cases showing architectural alteration in transverse/descending colon.

	Controls	CD	ITB	UC
None	30	22 (73.3%)	23 (76.7%)	12 (40%)
Mild	0	8 (26.7%)	5 (16.7%)	10 (33.3%)
Moderate	0	0	2 (6.7%)	7 (23.3%)
Severe	0	0	0	1 (3.3%)

Table 12: Proportion of cases showing chronic inflammation in transverse/descending colon.

	Controls	CD	ITB	UC
None	21	9 (30%)	7 (23.3%)	8 (26.7)
Mild	9 (30%)	11 (36.7%)	20 (66.7%)	7 (23.3%)
Moderate	0	10 (33.3%)	2 (6.7%)	11 (36.7%)
Severe	0	0	1 (3.3%)	4 (13.3%)

Table 13: Proportion of pyloric gland metaplasia, deep ulcers, fibrosis, pseudovillous change and atrophy in transverse/descending colon.

	Controls	CD	ITB	UC
Pyloric gland metaplasia	0	0	0	0
Deep Ulcer	0	5 (16.7%)	2 (6.67%)	7 (23.3%)
Fibrosis	2 (6.7%)	6 (20%)	4 (13.3%)	4 (13.3%)
Pseudovillous change	0	0	0	3 (10%)
Atrophy	0	0	0	2 (6.7%)

Table 14: Proportion of cases showing activity in transverse/descending colon.

	Controls	CD	ITB	UC
None	30	15 (50%)	21 (70%)	8 (26.7%)
Mild	0	10 (33.3%)	8 (26.7%)	12 (40%)
Moderate	0	5 (16.7)	1 (3.3%)	6 (20%)
Severe	0	0	0	4 (13.3%)

Table 15: Proportion of cases showing cryptitis in transverse/descending colon.

	Controls	CD	ITB	UC
None	30	19 (63.3%)	24 (80%)	8 (26.7%)
Mild	0	11 (36.7%)	5 (16.7%)	14 (46.7%)
Moderate	0	0	1 (3.3%)	8 (26.7%)

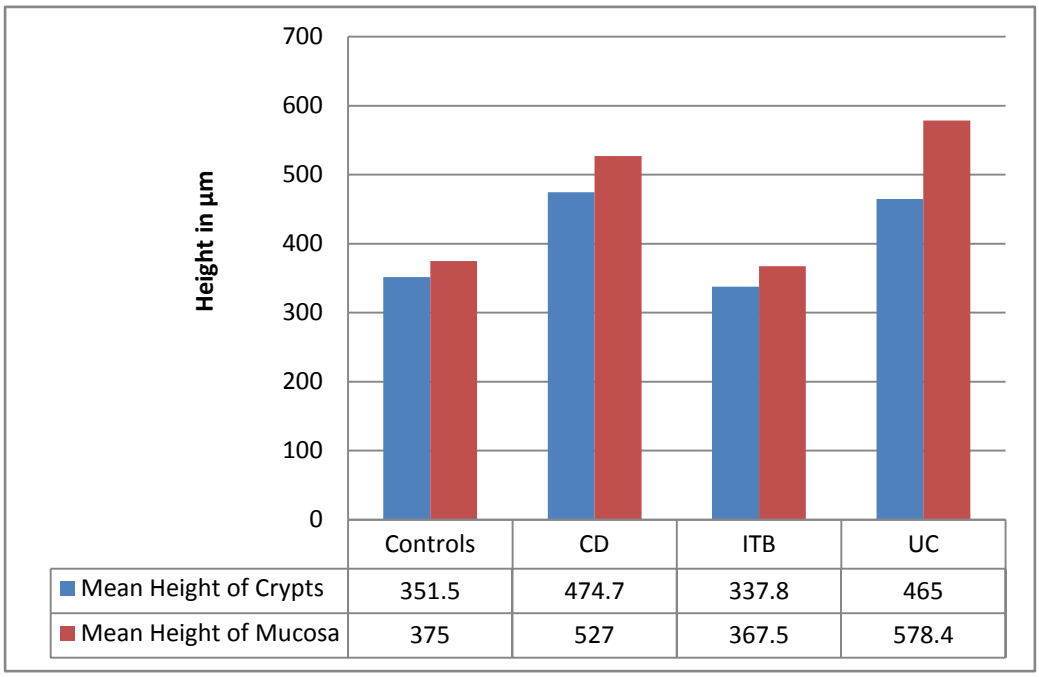

Figure 8: Morphometric measurements of transverse/descending colonic mucosa in diseases and controls (Expressed in µm).

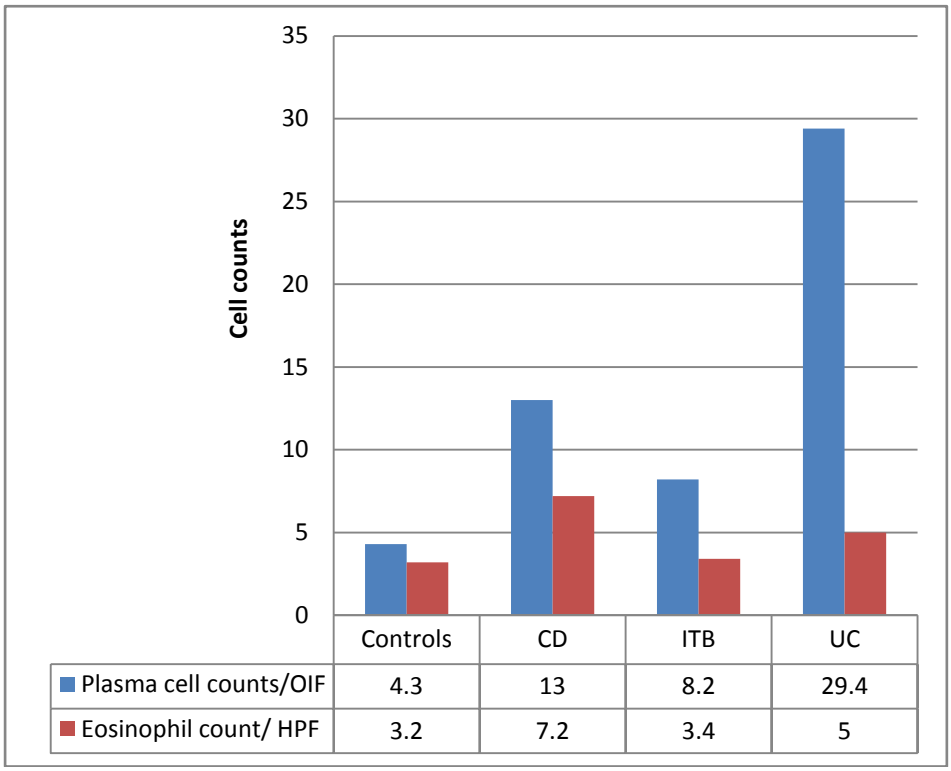

Figure 9: Mean cell counts in the transverse/ descending colon (Plasma cells /OIF, Eosinophils/HPF)

SIGMOID COLON/ RECTUM

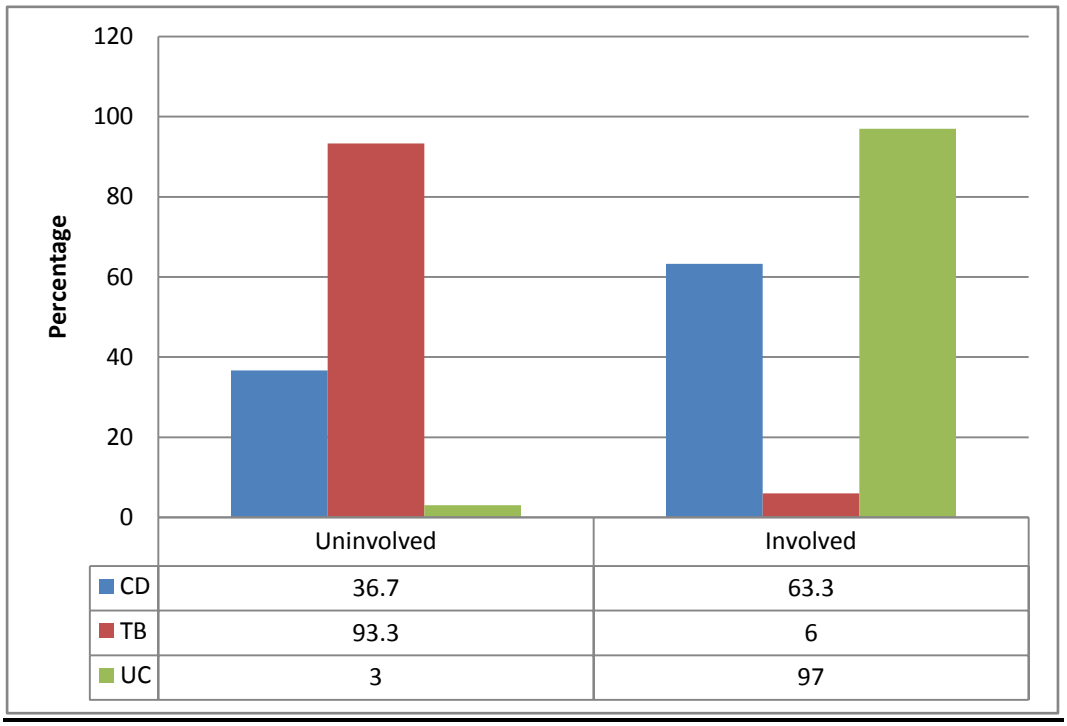

Figure 10: Proportion of sigmoid/rectum biopsies involved in disease.

Table 16: Proportion of cases showing architectural alteration in sigmoid/rectum biopsies.

	Control	CD	ITB	UC
None	29 (96.7%)	21 (70%)	28 (98.3%)	0
Mild	1 (3.3%)	5 (16.7%)	1 (3.3%)	6 (20%)
Moderate	0	4 (13.3%)	1 (3.3%)	14 (46.7%)
Severe	0	0	0	10 (33.3%)

Table 17: Proportion of cases showing chronic inflammation in sigmoid/ rectum biopsies.

	Control	CD	ITB	UC
None	28 (93.3%)	11 (36.7%)	26 (86.7%)	0
Mild	2 (6.7%)	9 (30%)	3 (10%)	1 (3.3%)
Moderate	0	9 (30%)	0	10 (33.3%)
Severe	0	1 (3.3%)	1 (3.3%)	19 (63.3%)

Table 18: Proportion of focal activity, paneth cell metaplasia, depp ulcers, fibrosis, pseudovillous change and atrophy in sigmoid/rectum.

	Controls	CD	ITB	UC
Focal activity	0	5 (17%)	3 (10%)	0
Paneth cell metaplasia	0	0	0	6
Deep Ulcers	0	0	1 (3.3)	22 (73.3%)
Fibrosis	7 (23.3%)	11 (36.7%)	3 (10%)	16 (53.3%)
Pseudovillous change	0	0	0	4 (13.3%)
Atrophy	0	0	0	14 (46.7%)

Table 19: Proportion of cases showing activity in sigmoid/rectum.

	Controls	CD	ITB	UC
None	29 (96.7%)	17 (56.7%)	25 (83.3%)	0
Mild	1 (3.3%)	10 (33.3%)	4 (13.3%)	1 (3.3%)
Moderate	0	3 (10%)	1 (3.3%)	25 (83.3%)
Severe	0	0	0	4 (13.3%)

Table 20: Proportion of cases showing cryptitis in sigmoid/rectum.

	Controls	CD	ITB	UC
None	29 (96.7%)	17 (56.7%)	28 (93.3%)	1 (3.3%)
Mild	1 (3.3%)	10 (33.3%)	2 (6.7%)	12 (40%)
Moderate	0	3 (10%)	0	17 (56.7)

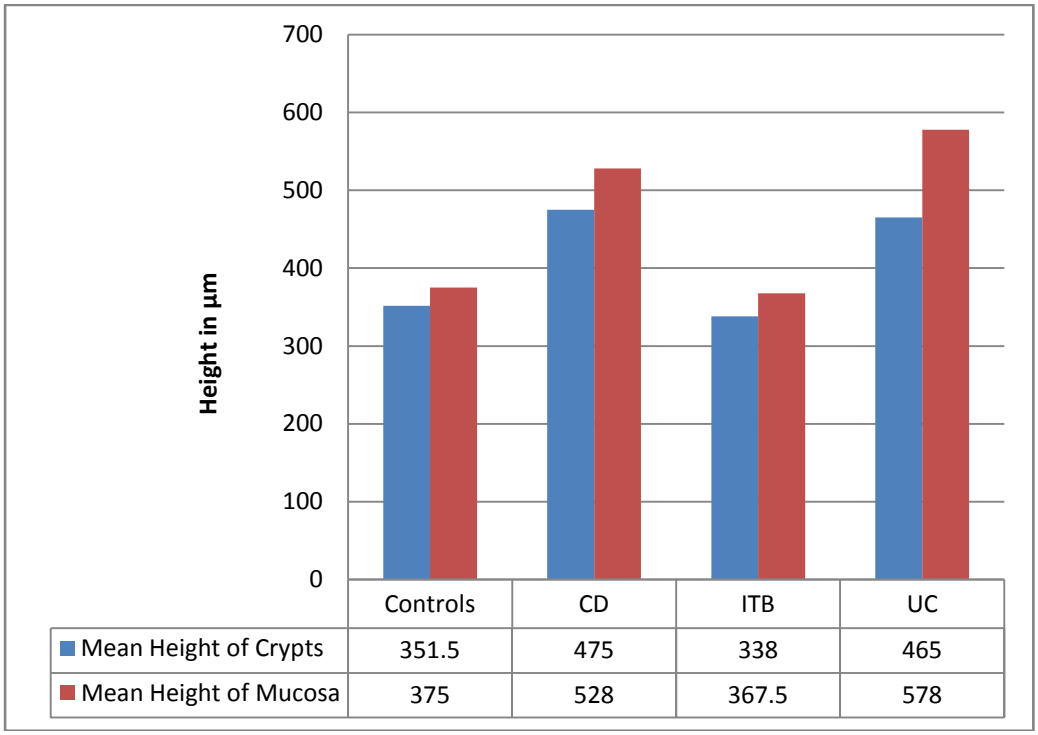

Figure 11: Morphometric measurements of sigmoid/rectal mucosa in diseases and controls (Expressed in µm).

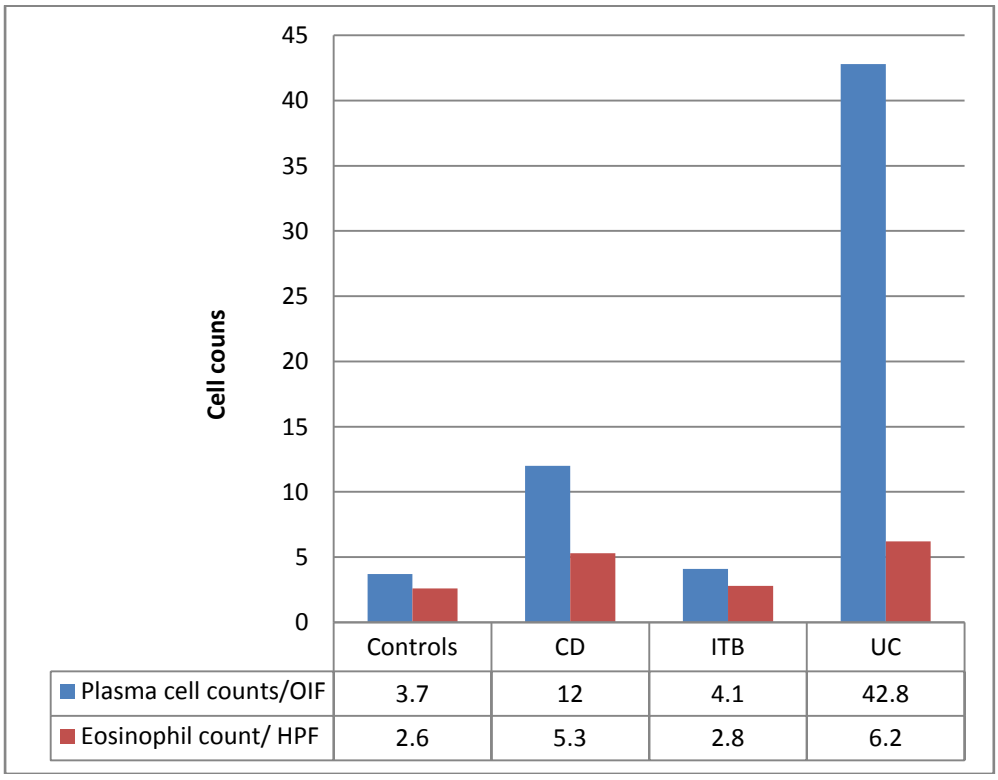

Figure 12: Mean cell counts in the sigmoid/ rectum (Plasma cells /OIF, Eosinophils/HPF)

Table 21: Salient features of Crohn's disease in comparison to intestinal TB in ileal biopsies.

Ileum	CD (n= 30)	ITB (n= 30)	p-value
Involved	87 %	83 %	**NS**
Architectural alteration	67 %	84 %	**<0.05**
Chronic inflammation	54 %	80 %	**<0.05**
Fibrosis	26 %	54 %	**<0.05**
Deep ulcers	30 %	67 %	**< 0.05**
Cryptitis	37 %	64%	**<0.05**
Avg. Villous height	298µm (40% decrease)	184µm (60% decrease)	**<0.001**
Avg. Mucosal height	590µm	539µm	**0.02**
Mean Plasma cell count/ OIF	14.4 (350% increase)	18.2 (460% increase)	**0.01**
Mean eosinophil count/ HPF	6.5 (200% increase)	4.2 (90% increase)	**0.002**
Granulomas	3 (10 %)	25 (83 %)	**-**
Mean dimension of granuloma	65.6µm	208µm	**-**
Largest granuloma	95µm	440µm	**-**
Necrotising granulomas	0	5	**-**

Table 22: Salient features of Crohn's disease in comparison to intestinal TB in caecum/ascending colon.

Caecum/ Ascending colon	CD (n= 30)	ITB (n= 30)	p-value
Involved	84%	80%	**NS**
Architectural alteration	70%	54%	**0.05**
Chronic inflammation	70%	47%	**<0.05**
Pyloric gland metaplasia	7%	0	**<0.05**
Fibrosis	17%	60%	**<0.001**
Deep ulcers	13	67	**<0.05**
Cryptitis	57%	73%	**0.05**
Mean Plasma cell count/ OIF	14.2 (113% increase)	23 (250%)	**<0.001**
Mean eosinophil count/ HPF	8.7 (55%)	7 (30%)	**0.01**
Granulomas	8 (27%)	25 (83%)	**-**
Mean dimension of granuloma	87.8µm	252.6µm	**-**
Largest granuloma	132.5µm	545µm	**-**
Necrotising granulomas	0	7	**-**

Table 23: Salient features of Crohn's disease in comparison to intestinal TB in rectum/ sigmoid.

Sigmoid colon/ rectum	CD (n= 30)	ITB (n= 30)	p-value
Involved	63%	7%	**<0.05**
Architectural alteration	30%	7%	**<0.05**
Chronic inflammation	64%	13%	**<0.05**
Cryptitis	54%	7%	**<0.05**
Fibrosis	37%	10%	**<0.05**
Avg. Mucosal height	527 µm (40% increase)	367 µm	**<0.001**
Mean Plasma cell count/ OIF	12(220% increase)	4.1	**<0.001**
Mean eosinophil count/ HPF	5.3	2.8	**NS**
Granulomas	5 (17%)	2 (7%)	**-**
Mean dimension of granuloma	90.6µm	228.2µm	**-**
Largest granuloma	115µm	412µm	
Necrotising granulomas	0	0	**-**

Table 24: Salient features of Crohn's disease in comparison to Ulcerative colitis in ileal biopsies.

Ileum	CD (n=30)	UC (n=30)	p-value
Ileal involvement	87%	10%	**<0.05**
Avg. Height of villi	298 (40% decrease)	422 (similar to controls)	**<0.001**
Plasma cells/ OIF	14.4 (350% increase)	4.2 (similar to controls)	**<0.001**
Eosinophils/ HPF	6.5 (200% increase)	2.4 (similar to controls)	**0.005**

Table 25: Salient features of Crohn's disease in comparison to UC in colon and rectum.

Colon and rectum	CD (n=30)	UC (n=30)	p-value
Rectal involvement	63%	97%	**<0.05%**
Rectal sparing	37%	3%	**<0.05**
Pyloric gland metaplasia	7%	0	**NS**
Paneth cell metaplasia	0	30%	**0.001**
Pseudovillous change	0	23%	**0.01**
Architectural alteration	30%	100%	**<0.001**
Moderate -severe chronic inflammation	33%	96%	**<0.001**
Focally enhanced colitis	17%	0	**-**
Ulcers in rectum	0	76%	**-**
Crypt abscesses	7%	77%	**<0.05**
Crypt distortion	5%	21%	**<0.001**
Crypt branching	1.3%	4.8%	**<0.001**
Fibrosis	37%	54%	**NS**
Crypt atrophy	0	47%	**<0.001**
Granulomas	46%	0	**<0.001**
Mucosal height	527µm	578µm	**NS**
Mean Plasma cell count/ OIF	12 (220% increase)	42.8 (1000% increase)	**<0.001**
Mean esinophil count/ HPF	5.3	6.0	**NS**

RESULTS

HISTOLOGICAL FEATURES AND MORPHOMETRY

CONTROLS

Ileum

The ileal mucosa was within normal limits in all the control cases. The crypt villous architecture was normal in all. 93% of the control ileal biopsies showed no significant inflammation, and 7% showed mild chronic inflammation. None of the control cases showed evidence of activity, deep ulceration, cryptitis or crypt abscesses. Pyloric metaplasia was not seen, but fibrosis was seen in 13%.

The mean mucosal height in the ileum of controls was 569.13±27.2µm, with the mean height of villi being 475.47±30.98µm. The mean plasma cell count in the deep mucosa was 3.2±1.5/ OIF and the mean eosinophil count per HPF was 2.3±0.8.

Cecum/ascending colon

The caecum/ascending colonic mucosa was also normal in all cases. None of the cases showed any architectural alteration. 9 cases (30%) showed mild chronic inflammation and one case showed focal activity. There was no evidence of more severe chronic inflammation or activity including ulcers, cryptitis or crypt abscesses or any other evidence of chronic colitis including fibrosis, pyloric metaplasia, pseudovillous change or atrophy.

The mean height of the caecum/ascending colonic mucosa in controls was 333.33±21.45µm and that of the crypts was 321.80±19.02 µm. The mean plasma cell

count in the deep mucosa was 6.6±1.3 per OIF. The mean eosinophil count was 5.6±1.3 per HPF.

Transverse/descending colon

The mucosa of the transverse/descending colon was also normal in all cases. None of the cases showed any architectural alteration, metaplasia, pseudovillous change or atrophy. 9 cases (30%) showed mild chronic inflammation, but there was no evidence of more severe chronic inflammation, activity, ulcers, cryptitis or crypt abscesses. There was fibrosis in 2 cases.

The mean height of the mucosa from the transverse/descending colon in controls was 343.93 ± 22.94µm and that of the crypts was 322.47± 24.32 µm. The mean plasma cell count in the deep mucosa was 4.3±1.6 per OIF. The mean eosinophil count was 3.2±1.1 per HPF.

Sigmoid/rectum

The mucosa of the recto-sigmoid in some of the control cases showed mild changes. There was mild architectural alteration in one case, but no evidence of 73aseou cell metaplasia, pseudovillous change or atrophy. 2 cases (7%) showed mild chronic inflammation and 1 cases showed mild activity and mild cryptitis. Ulcers or crypt abscesses were not seen. There was fibrosis in 7 cases (23%).

The mean height of the recto-sigmoid mucosa in controls was 375.07 ± 53.05 µm and that of the crypts was 351.53 ± 50.19µm. The mean plasma cell count in the deep mucosa was 3.7±1.3 per OIF. The mean eosinophil count was 2.6±1.2 per HPF.

CROHN'S DISEASE

Ileum

The ileum was involved in 87% of the CD cases (mild in 27%, moderate/severe in 60%). Ileal sparing was seen in 4 cases (13%) of which 2 showed changes in all the colonic segments, and 2 showed changes only in the proximal and mid colon (with rectal sparing). Architectural alteration was seen in 20 ileal biopsies (40% mild, 27% moderate) in CD and pyloric metaplasia in 5 cases.

Moderate or severe chronic inflammation were seen in 16 (53% - 50% moderate, 3% severe). Fibrosis was seen in 8 biopsies. Granulomas were seen in only 3 of the ileal biopsies. The mean number of granulomas per biopsy with granulomas was 1.6. The mean diameter of the granulomas was 65.6µm and the largest granuloma was 95 µm.

Among the different segments studied, activity was most frequent in the ileum and was seen in 22 cases (73%)(43% mild, 30% moderate). Deep ulceration was seen in 9 biopsies. Cryptitis was seen in 11 ileal biopsies and crypt abscesses in 3. More than 2 crypt abscesses were not seen in any of the cases. Focal activity was seen in only 1 biopsy.

The mean ileal mucosal height did not change significantly in biopsies of patients with CD. The mean height of villi, on the other hand, was significantly reduced from 475.47±30.98µm in the controls to 298µm (40% decrease). The mean plasma cell count in the deep ileal mucosa was 14.5±9.4/ OIF. The greatest increase in eosinophil counts was in CD and the segment with the greatest increase was the ileum where the

increase was almost three-fold (increase from 2.3±0.8/OIF to 6.5±2.9 /OIF in all involved segments).

Cecum/ascending colon

The caecum/ascending colon was involved in 83% of the CD cases (mild 43%, moderate 40%). Architectural alteration was seen in 16 biopsies (54% - 37% mild, 17% moderate) and pyloric metaplasia was seen in 2 cases. Atrophy was seen only in 1 biopsy.

Moderate or severe chronic inflammation were seen in 14 cases (46% - 33% moderate, 13% severe). Fibrosis was seen in 5 cases.

Granulomas were seen in 8 of the caecal/ascending colonic biopsies. The mean number of granulomas was 1.8 in the cecum/ascending colon and mean diameter of the granulomas was 87.8 µm and largest granuloma was 132.5 µm.

Activity was seen in 21 biopsies (70%) from the cecum/ascending colon (47% mild, 23% moderate), deep ulceration in 4 biopsies and superficial ulceration in 1. Cryptitis was seen in 17 biopsies form the caecum/ascending colon and crypt abscesses were seen in 14 biopsies. More than 2 crypt abscesses were not seen in any of the biopsies from the cecum/ascending colon. Focal activity was seen in 2 biopsies.

The mean height of the caecum/ascending colonic mucosa in CD was 482.9±98µm and the mean height of the crypts was 418µm (controls 321.80±19.02). The mean

plasma cell count in this site was 14.19±4.9/ OIF. The mean eosinophil count was 8.7±1.8/ HPF. Crypt distortion was seen in 10.13±11.83% of all crypts in diseased mucosa, whereas crypt branching was seen in only 3.33±3.27%.

Transverse/descending colon

The transverse colon was involved in 19 (70%) of the CD cases (43% mild, 27% moderate). Architectural alteration was seen in 8 biopsies (27% - all mild). Pseudovillous change was seen in only 1 biopsy.

Activity was seen in 15 biopsies (50%) from the transverse/descending colon (33% mild, 27% moderate). Deep ulceration was seen in 5 biopsies from the transverse/descending colon. Cryptitis was seen in 11 biopsies and crypt abscesses in 8. More than 2 crypt abscesses were seen in 1 biopsy from the transverse/descending colon. Focal activity was seen in 3 biopsies. Moderate or severe chronic inflammation was seen in 10 biopsies (33% - all moderate). Fibrosis was seen in 6 biopsies.

Granulomas were seen in 9 of the transverse/descending colon biopsies. The mean number of granulomas was 1.6 per site and the mean diameter of the granulomas was 88.5 µm. The largest granuloma measured 145 µm.

The mean height of the transverse/descending colon mucosa in CD was 487.7±117µm and the mean height of the crypts was 433.8±100.4µm.

The mean plasma cell count in the transverse/descending colonic biopsies was 12.8±6.6/ OIF. The mean eosinophil count was 7.2±3.8 and 5.3±3.5/ OIF. Crypt distortion was seen in 5.98±4.30 of all crypts in diseased mucosa, whereas crypt branching was seen in only 1.23±1.07 %.

Sigmoid/rectum

The recto-sigmoid was involved in 63% of the CD cases (mild 40%, moderate 23%). A total of 11 cases (37%) showed rectal sparing. Architectural alteration was seen in 9 biopsies (30% - 17% mild, 13% moderate).

Moderate or severe chronic inflammation were seen in 10 (33% - 30% moderate, 3% severe). Fibrosis was seen in 11 biopsies from the recto-sigmoid.

Granulomas were seen in 5 of the recto-sigmoid biopsies. The mean number of granulomas was highest in the recto-sigmoid being 2.4/site. The mean diameter of the granulomas was also the highest in the recto-sigmoid: 90.6 µm. The largest granuloma was 115.5 µm.

Activity was seen in 13 biopsies (43%) from the recto-sigmoid (33% mild, 10% moderate). Deep ulceration was not seen in any of the recto-sigmoid biopsies, but superficial ulcers were seen in 2. Cryptitis was most frequent in the recto-sigmoid and was seen in 16 biopsies. Crypt abscesses were seen in 8 biopsies and more than 2

crypt abscesses were seen in 2 biopsies from the recto-sigmoid. Focal activity was most frequent in the recto-sigmoid and was seen in 5 cases.

The mean height of the recto-sigmoid colonic mucosa in CD was 527.7 ±101µm and the mean height of the crypts was 474 ±80 µm. The mean plasma cell count was 12±4.8/ OIF. Only 13 cases showed a mean plasma cell count of >10/ OIF.

The mean eosinophil count was 5.3±3.5 per HPF. Branching of crypts was seen in only 1.31± 1.26% whereas distortion was seen in 5.17±3.85 % of crypts in the diseased fragments.

Correlation with endoscopic findings:

Moderate/severe chronic inflammation and/or architectural alteration were seen in 49 of 59 biopsies from endoscopically involved segments (41 chronic inflammation; 39 architectural alteration) and 9 of 61 biopsies (chronic inflammation in 5 and architectural alteration in 7) from endoscopically uninvolved segments. Only chronic inflammation without architectural alteration was seen in 9 of the involved segments and 2 of the uninvolved. Only architectural alteration was seen in 8 of the 59 involved segments and 4 of the uninvolved segments.

Granulomas were seen in 15 of the 59 involved segments (14 cases) and 8 of the uninvolved segments (5 cases – of which in 4, granulomas were only seen in the uninvolved segments). Granulomas were associated with significant chronic

inflammation in 1/3, 3/7, 3/8 and 2/5 biopsies from the 4 proximal to distal segments. They were associated with architectural alteration in 3/3, 4/7, 3/8 and 3/5 of the proximal to distal segments.

TUBERCULOSIS

Ileum

The ileum was involved in 83% of the TB cases (mild in 13%, moderate/severe in 60%). Ileal sparing was seen in 5 cases (17%) of which 2 showed changes in only the cecum/ascending colon, 2 in the cecum to descending colon and one exclusively in the recto-sigmoid biopsy.

Architectural alteration was seen in 25 ileal biopsies (57% mild, 23% moderate, 3% severe) and pyloric gland metaplasia was present in 8 biopsies.

Moderate or severe chronic inflammation was seen in 80% (73% moderate, 7% severe). Fibrosis was seen in 16 biopsies.

Granulomas were seen in 25 biopsies from the ileum. The mean number of granulomas per biopsy with granulomas was 2 and the mean diameter of the granulomas was 208.2 µm. The largest granuloma was 440 µm in diameter.

Among the different segments studied, activity was most frequent in the ileum and was seen in 26 cases (87%) (23% mild, 63% moderate). Deep ulceration was seen in 20 biopsies and superficial ulcers in 2. Cryptitis was seen in 19(63%) ileal biopsies

(57% mild, 7% moderate) but crypt abscesses in only 2 (7%) cases with 2 and 3 crypt abscesses each. Focal activity was seen in 2biopsies.

The mean ileal height was 539.8 ± 97.2 µm. The mean ileal mucosal height did not change significantly from that of controls. The mean height of villi, on the other hand, was significantly reduced from 475.47±30.98µm in the controls to 183.8±101.4µm. The mean plasma cell count in the deep ileal mucosa was 18.1±6.7/ OIF. The mean eosinophil count was 4.2±1.9/ HPF.

Cecum/ascending colon

The cecum/ascending colon was involved in 80% of the ITB cases (mild 20%, moderate 60%). Architectural alteration was seen in 21 biopsies (70%) (53% mild, 17% moderate). Pyloric metaplasia, atrophy and pseudovillous changes were not seen in any biopsy.

Moderate or severe chronic inflammation was seen in 21 (70%) (47% moderate, 23% severe). Fibrosis was seen in 18 biopsies.

25 biopsies from the caecum/ascending colonic mucosa had granulomas. The mean number of granulomas was 2.6 and the mean diameter of the granulomas was 252.6 µm. The largest granuloma measured 545 µm in diameter.

Activity was seen in 26 biopsies (87%) from the cecum/ascending colon (23% mild, 60% moderate, 3% severe. Deep ulceration was seen in 20 biopsies and superficial ulceration in 1. Cryptitis was seen in 22 biopsies form the caecum/ascending colon and crypt abscesses were seen in 10 cases. More than 2 crypt abscesses were seen in only 1 biopsy from the cecum/ascending colon, which had 4 crypt abscesses. Focal activity was seen in 2 biopsies.

The mean height of the caecal/ascending colonic mucosa in ITB was 498.9±68.2µm and the mean height of the crypts was 423±47µm. The mean plasma cell count in the caecum/ascending colon was 22.7±8.1/ OIF (50% higher than in CD which was 14.19±4.9/ OIF). The mean eosinophil count was 7.4±1.3/ HPF. Crypt distortion was seen in 4.40±2.97% of all crypts in diseased mucosa, whereas crypt branching was seen in only 1.20±0.87%.

Transverse/descending colon

The transverse colon was involved in 9 (30%) of the ITB cases (20% mild, 10% moderate). Architectural alteration was seen in 7 biopsies (17% - mild; 7% moderate). Metaplasia, pseudovillous change and atrophy were not seen.

Moderate or severe chronic inflammation were seen in 3 cases (10%) (2 biopsies-moderate, 1 biopsy –severe). Fibrosis was seen in 4 cases.

Activity was seen in 9 cases (30%) from the transverse/descending colon (27% mild, 3% moderate). Deep ulceration was seen in 2 cases and superficial ulceration in 1 case. Cryptitis was seen in 6 cases (5 mild, 1 moderate) and crypt abscesses was seen in 3 cases. More than 2 crypt abscesses were not seen in any case. Focal activity was seen in 3 cases.

4 biopsies from the transverse/descending had granulomas. The mean number of granulomas was 1.8 per site and the mean diameter of the granulomas was 322.7µm. The largest granuloma measured 475 µm in diameter.

The mean height of the transverse/descending colonic mucosa in ITB was 375.27± 54.08µm and the mean height of the crypts was 340.40±43.94µm.

The mean plasma cell count in the transverse/descending colon was 8.2±6 OIF. The mean eosinophil count was 3.4±1.3/ HPF. Crypt distortion was seen in 2.9% of all crypts in diseased mucosa, whereas crypt branching was seen in only 0.7% cases.

Sigmoid/rectum

Involvement of the recto-sigmoid was rare in ITB (3% biopsies mildly and 3% moderately involved). A total of 28 cases (93%) showed rectal sparing. Architectural alteration was seen in 2 biopsies (7%) (1 mild, 1 moderate)

Severe chronic inflammation was seen in 1 case and moderate inflammation was not seen in any case. Fibrosis was seen in 3 biopsies.

Granulomas were seen in 2 of the recto-sigmoid biopsies. The mean number of granulomas was 4.5 in the recto-sigmoid. The mean diameter of the granulomas was 228.2µm and the largest granuloma measured 412.5 µm.

Activity was seen in 5 biopsies (17%) from the recto-sigmoid (13% mild, 3% moderate). Deep ulceration was seen in one biopsy and superficial ulcers were not seen. Mild cryptitis was seen in 2 biopsies and 1 crypt abscess in 1 biopsy. Focal activity was seen in 3 cases.

The mean height of the recto-sigmoid mucosa in ITB was 367.47 ±48.63µm and the mean height of the crypts was 337.80 ±40.45µm. The mean plasma cell count was 4.1± 3.4/ OIF. None of the ITB cases had more than 10 plasma cell count/ OIF except for those 2 cases which had granulomatous inflammation in the sigmoid/rectum.

The mean eosinophil count was 2.8/ HPF. Branching of crypts was seen in only 2.4% whereas distortion was seen in 5.5% of all crypts in the diseased fragments.

Granulomas were seen in all cases of ITB. They were seen in 56 of 120 biopsies: 25 biopsies from the ileum, 25 from the caecum/ascending, 4 from the transverse/descending and 2 from the recto-sigmoid. Granulomas were found in the terminal ileum and/or cecum/ascending colon in 29 cases and exclusively in the

rectosigmoid in only one case. 8 cases showed granulomas with central 84aseous necrosis.

Granulomas were associated with architectural alteration in 50 of 56 sites and chronic inflammation in 47. In the ileum, only one biopsy with a granuloma did not show significant chronic inflammation. All showed architectural alteration. In the cecum 4 of 25 sites with granulomas did not show chronic inflammation, in the transverse/descending colon 3 of 4 did not show and in the recto-sigmoid, 1 of 2 did not show chronic inflammation. In the ileum, all sites with granulomatous inflammation showed architectural alteration, in the cecum, 20 of 25 showed, in the transverse/descending 3 of 4 showed and in the recto-sigmoid, both cases showed architectural alteration.

Correlation with endoscopic findings

65 biopsies were from endoscopically involved segments, of which 54 had granulomas. Biopsies from distal segments involved by disease were less likely to show granulomas (3 of 8 from the transverse colon and 2 of 5 from the recto-sigmoid) compared to biopsies from the proximal segments (25/26 in the ileum and 24/26 in the cecum/ascending colon. Among 55 biopsies from the uninvolved segments, only one showed a granuloma.

Granulomas were found in only 2 biopsies from sites that were uninvolved by disease on endoscopy, and in both these there was architectural alteration. Severe chronic inflammation was also present in one of them.

ULCERATIVE COLITIS

Ileum

The ileum was involved in only 10% of the UC cases and the changes were mild. Architectural alteration was seen in 5 cases (17%) (mild in 10%, moderate in 7%) and pyloric metaplasia in 1 case only. Moderate chronic inflammation was seen in 4 biopsies (13%) but fibrosis was seen in 7 biopsies. Mild activity was seen in 5 cases (17%) and none of the cases showed deep ulcers or crypt abscesses in the ileum. Mild cryptitis was seen in 2 biopsies. Focal activity was seen in 1 biopsy.

The mean ileal mucosal height (591.80± 41.77µm) in biopsies of patients with UC was not significantly different from that in controls. The mean height of villi (422.87± 57.89µm) also was not significantly altered. The mean plasma cell count in the deep ileal mucosa was 4.2±3.9/ OIF and the mean eosinophil count was 2.4±0.9/ HPF.

Cecum/ascending colon

The cecum/ascending colon was involved in 30% of the UC cases (mild 27%, moderate 3%). Architectural alteration was seen in 8 biopsies (mild alteration in 23% and moderate in 3%). Atrophy was not seen in any of the biopsies from the cecum/ascending colon. Moderate chronic inflammation was seen in 3 biopsies (10%). Fibrosis was also seen in 3 biopsies. Activity was seen in 16 biopsies (53%) from the cecum/ascending colon (43% mild, 10% moderate) with deep ulceration in 1 biopsy and superficial ulceration in 1. Mild cryptitis was seen in 15 biopsies from the

cecum/ascending colon and crypt abscesses were seen in 3 (2 with 1 crypt abscess each and 1 with 3 crypt abscesses). Focal activity was seen in 8 biopsies (27%).

The mean height of the caecal/ascending colon mucosa in UC was 450± 108µm and the mean height of the crypts was 402±88.6µm (controls 321.80±19.02). The mean plasma cell count in the caecum/ascending colon was 18.8±16/ OIF. The mean eosinophil count was 6.4±2.3/ HPF. Crypt distortion was seen in 17.97±20.07% of all crypts in diseased mucosa, whereas crypt branching was seen in only 6.99±14.18%.

Transverse/descending colon

The transverse colon was involved in 16 of the UC cases (27% mild, 27% moderate). Architectural alteration was seen in 18 biopsies (33% mild; 26% moderate or severe). Paneth cell metaplasia was seen in 3 cases, pseudovillous change in 3 cases and atrophy in 2 cases.

Moderate or severe chronic inflammation were seen in 15 cases (50%) (37% moderate, 13% severe). Fibrosis was seen in 4 cases.

Activity was seen in 22 biopsies (73%) from the transverse/descending colon (40% mild, 20% moderate, 13% severe). Deep ulceration was seen in 7 biopsies and superficial ulceration in 1. Cryptitis was also seen in 22 biopsies (73%) with mild cryptitis in 47% and moderate in 27%. Crypt abscesses were present in 15 biopsies.

More than 2 crypt abscesses were seen in 11 biopsies from the transverse/descending colon. Focal activity was seen in 4 biopsies.

The mean height of transverse/descending colonic mucosa in UC was 522±99µm and the mean height of the crypts was 427±71µm. The mean plasma cell count in the transverse/descending colon was 29.4±12.8/ OIF. The mean eosinophil count was 4.9±1.9/ HPF. Crypt distortion was seen in 13.89±11.55 of all crypts in diseased mucosa, whereas crypt branching was seen in only 2.77±1.86 %.

Sigmoid/rectum

The recto-sigmoid was involved in 100% of the UC cases (mild 13%, moderate 87%).

Architectural alteration was seen in all biopsies (20% mild, 47% moderate, 33% severe. Paneth cell metaplasia was seen in 6 cases (20%), pseudovillous change in 4 (13%) and atrophy in 14 cases (47%). Moderate or severe chronic inflammation were seen in 29 biopsies (33% moderate, 63% severe). Fibrosis was seen in 16 biopsies (53%) from the rectosigmoid.

Activity was seen in all biopsies from the recto-sigmoid (3% mild, 83% moderate, 13% severe). Deep ulceration was seen in 22 biopsies and superficial ulceration in 3. Cryptitis was most frequent in the recto-sigmoid and was seen in 29 biopsies (mild in 40%, moderate in 57%). Crypt abscesses were also seen in 29 biopsies and more than

2 crypt abscesses were seen in 23 biopsies from the recto-sigmoid. Focal activity was not seen.

The mean height of the recto-sigmoid mucosa in UC was 578.33 ± 89.85µm and the mean height of the crypts was UC 463.47±86.77µm. The mean plasma cell count was 42.8±15.4/ OIF. The mean eosinophil count was 6.2±2.9/ HPF. Branching of crypts was seen in only 4.86±2.57 whereas distortion was seen in 20.78±13.45 of all crypts in the diseased fragments.

COMPARISON BETWEEN DISEASE GROUPS

CD versus ITB

ITB cases showed a marked proximal predominance of involvement by disease and distal sparing. Lesions were restricted to the ileum or cecum/ascending colon in 20 cases of TB and the rectum was spared in 28 of 30 cases. The mean plasma cell count was less than 10/ OIF in all cases with rectal sparing.

TB showed a higher prevalence of ulceration, architectural alteration and activity in the ileal and proximal colonic segments than the mid and distal colonic segments as compared to CD. Rectal sparing was seen in only one third of the CD cases. Lesions were restricted to the ileum or caecum/ascending colon in only 7 cases of CD. In 25 cases of CD the mean plasma cell count was more than 10/ OIF in the recto-sigmoid mucosa.

Granulomas were seen in all cases of ITB. They were frequently located in the ileum and cecum (25 cases each) and rarely in the mid or distal colon (4 and 2 cases respectively). Granulomas were large (>400µm) in 11 cases and >4 granulomas were seen per segment in 7 cases of TB, but in none of the cases of CD. Granulomas were associated with deep ulceration in 29 of 30 cases of TB, but only 2 cases of CD. Granulomas were rarely seen in uninvolved sites.

In CD, granulomas were rare in the ileum (3 cases) compared to the colon (8, 9 and 5 cases from the proximal, mid and distal colon) and the largest granulomas and highest numbers were seen in the recto-sigmoid. In our study, all granulomas were less than 200µm. No site had more than 4 granulomas.

Granulomas were often seen in endoscopically uninvolved sites (8 out of 25 sites) and were not associated with other significant chronic inflammatory changes (moderate or severe chronic inflammation, architectural alteration or deep ulceration) in 6 sites from 5 cases. No case had necrotising granulomas whereas 26% of the ITB cases showed necrotising granulomas.

1/3rd of granulomas in CD are seen in endoscopically uninvolved sites. Whereas 2 out of 56 sites with granulomas were endoscopically uninvolved.

In the ileal biopsies with moderate/severe chronic inflammation, the mean mucosal height and villous height were significantly more in CD than in ITB (mucosal height: 590±55µm and 539±97µm; villous height: 298±72µm and 183±101µm) (P-values

0.028 and 0.032). The mean eosinophil count was also significantly higher in CD than ITB (mean eosinophil count/ HPF: 6.5 and 4.2 in CD and ITB (P-value = 0.002). There was no significant difference in the mean plasma cell count/ OIF.

Crypt distortion was more common in CD (percentage of branched crypts: (P-value 0.02). The eosinophil count was also significantly higher in CD (P-value < 0.001), but the plasma cell count/ OIF showed a reverse trend with a higher mean in ITB as compared to CD, both in the ileum(18 vs 14) and caecum (22 vs 14) (P-value .011).

Moderate or severe chronic inflammation was also more frequent in the proximal segments in ITB and in the distal segments in CD (24,21,3,1 in ITB and 16,14,10,10 in CD), but the prevalence of architectural alteration was similar in the proximal segments but higher in the distal segments in CD (25,21,7,2 in TB and 26,25, 21 and 19 in CD). TB biopsies showed more frequent fibrosis in the proximal segments (16 cases in ileum,18 in caecum/ascending colon, 4 in transverse/descending and 3 in recto-sigmoid) whereas fibrosis was more frequent in the distal segments in CD (6 cases in transverse/descending and 11 in recto-sigmoid) .

Deep ulceration (20 cases in ileum, 20 cases in caecum/ascending of ITB whereas 9 cases in ileum and 4cases in caecum/ascending of CD), moderate/severe activity (19 in ileum and 19 in right colon of ITB where as 9 in ileum and 7 in right colon of CD) and cryptitis (19 in ileum and 22 in right colon of ITB whereas 11 in ileum and 17 in right colon of CD) were twice as frequent in the proximal sites in ITB. In the mid and

distal colon they were slightly more frequent in CD. The prevalence of focal activity was not very different in ITB and CD.

In cases of TB, the presence of granulomas>400µm, >4 granulomas per segment, deep ulceration at the site of granulomatous inflammation and rectal sparing could identify 100% of cases.

In cases of CD, the absence of large granulomas, <5 granulomas per segment, absence of deep ulceration at the site of granulomatous inflammation and rectal involvement by disease could identify 28 of 30 cases. 2 cases showed deep ulcers and granulomas at the same site, but both of these showed rectal involvement by disease.

CD versus UC

The changes in CD decreased in intensity from the ileum to the rectum (26,25,21 and 19 biopsies were involved from the ileum to the rectum, respectively) whereas those in UC were the reverse (3,9,16,29 from ileum to rectum, respectively). The ileum was involved in only 3 cases of UC and only mild changes were present unlike the 26 cases (8 mild, 18 moderate) from patients with CD. The rectum was spared in 11 cases of CD, but only in 1 cases of UC

The prevalence of chronic inflammation and architectural alteration also showed similar trends (architectural alteration: 20,16,8 and 9 in CD and 5,8,18 and 30 in UC; chronic inflammation:16,14,10,10 in CD and 4,3,15 and 29 in UC), as did the

presence of deep ulceration (9,4,5,0 in CD and 0,1,7,22 in UC) and activity (22,21,15,13 in CD and 5,16,22,30 in UC).

Pyloric metaplasia was seen in 5 and 2 cases from the ileum and caecum/ascending colon in CD, but only 1 case of UC in the ileum. Paneth cell metaplasia was seen in 3 cases from the transverse/descending colon and 6 cases in the recto-sigmoid in UC, but in none of the cases of CD. Atrophy and pseudovillous change were rare in CD (1 cases each in the caecum/ascending colon and transverse/descending colon) and more common in UC (atrophy in 2 and 14 from mid and distal colon; pseudovillous change in 1, 3 and 4 biopsies from the proximal, mid and distal colon).

Crypt abscesses were more frequent in UC than CD. They were seen in 2, 10, 3 and 1 biopsy from ileum till rectum in CD, but in 0, 3, 15, 29 biopsies from the proximal to the distal segments in UC. More than two crypt abscesses in a site were seen in 12 biopsies from the transverse/descending colon (40%) and 23 biopsies from the rectosigmoid (77%) in UC, but only 1 from the caecum and transverse and 2 from the recto-sigmoid in CD.

Mean plasma cell counts were 2 to 3 fold higher in the histologically involved distal colon in ulcerative colitis when compared with CD. The mean plasma cell count in biopsies from all 3 segments that showed histological involvement in UC were 18.8, 29.4 and 42.8 / OIF from caecum to rectum, whereas they were 14.19, 12.8 and 12 in CD. (P-value < 0.05)

The greatest increase in eosinophil counts was in ileum of patients with CD where the increase was almost three-fold when compared with controls. The mean count decreased distally in the colon and was 8.7, 7.2 and 5.3 in the caecum/ascending colon, transverse/descending and recto-sigmoid. In UC, the mean eosinophil count was similar in all the segments, being 6.4±2.3, 4.9±1.9 and 6.2±2.9 respectively in the caecum/ascending, transverse/descending and recto-sigmoid.

The percentage of distorted crypts reflected trends in architectural alteration and were highest in UC as compared to CD. (10.1%, 5.9%, 5.2% from the proximal to the distal colon in CD whereas 17.97, 13.89, 20.78% in UC). (P-value < 0.001)

Granulomas were present in 17cases of CD. They were not seen in any of the cases of UC.

Inter-observer correlation

The inter-class correlation coefficient (ICC) was calculated for the plasma cell count and eosinophil count on 40 cases (10 from each group). The inter observer variability for plasma cells was 0.93 (range 0.86 to 0.96) and the inter observer variability for eosinophils was 0.87 (range 0.81-0.94)

DISCUSSION

PATIENT DEMOGRAPHY

Our study included 30 normal controls and 30 clinically confirmed cases of Crohn disease, Ulcerative colitis and intestinal Tuberculosis. Out of the 30 cases of CD 19 were males (63%) and 11 were females (37%) with a male to female ratio of 1.7:1 and mean age of 37.4 years (range 14 to 63 years). This finding of male preponderance and younger age is in correlation with recent studies on CD published from India, including 2 studies from our own institute[19,92].A study by Das et al, which was a large collaborative study from 3 tertiary institutes of our country (AIIMS –Delhi, SGPGI-Lucknow and IPGMER-Kolkata) also reported similar findings with male to female ratio of 1.9:1 and mean age of patients being 34.5 years. A relative male predominance is however, uncommon in the west[29,98]. This finding from our study could possibly be explained by the fact that women approach hospitals for medical attention less frequently than men. This male preponderance has been reported in virtually all of the studies from India and other Asian countries as well[29,99].

Out of the 30 cases of intestinal tuberculosis(ITB) 18 were males (60%) and 12 were females (40%) with a mean age of 35.8 years (range 17 to 57 years).A study from south Africa by Kirsch et al also shows similar findings[30]. However, recently a few studies have shown a relative female predominance for ITB [14,33].

The mean age and male sex predominance were similar in both CD and ITB in our study.

Out of the 30 cases of Ulcerative colitis, 21 cases were males (70%) and the remaining 9 cases were females (30%) with a mean age of 40 (range 19 to 72 years). This is consistent with existing literature[1,3,5].

ENDOSCOPY

In our study we found that the endoscopic features that were commonly seen in cases of CD as compared to ITB and UC were the presences of aphthous ulcers, cobblestone appearance of mucosa, serpiginous linear ulcers, skip lesions, peri-anal disease (fistula, fissure, sinuses) and involvement of more than 4 anatomical sites. Endoscopic features that were more common in cases of intestinal TB were involvement of less than 4 anatomical sites, ulcers in the ileum and caecum and a deformed ileo-caecal valve. In UC it was the presence of ulcers in the rectum and sigmoid colon(93%), continuous disease and presence of pseudopolyps. In our study no case of ITB or UC showed a cobblestone appearance or serpiginous ulcers and no case of intestinal TB or CD showed the presence of pseudopolyps. None of our UC cases had perianal disease, skip lesions, cobble stone appearance or a deformed ileo-caecal valve. Our findings are consistent with the finding of Lee et al[14] who proposed that cobble stoning, aphthous ulcers, longitudinal ulcers and ano-rectal lesions were higher in cases of CD as compared with intestinal TB. In their study features in favour of ITB were less than 4 anatomically involved sites, deformed ileo-caecal valve, transverse ulcers and scars or pseudopolyps. Makharia et al[33] have also reported similar findings in their study. The frequency of ileo-caecal valve involvement of CD and ITB in our study was

similar to that in the studies by Lee et al and Makharia et al. However both these studies have not specifically mentioned any details about ileal ulceration. In our study we found ileal ulceration was present endoscopically in 83% cases of ITB as compared to 43% of CD.

NORMAL CONTROLS

In our study we had chosen those cases as normal controls in which the colonoscopy was reported as normal study and the biopsy also showed no specific lesion. We found none of the ileal biopsies to be abnormal in any of the 30 biopsies. The normal villous-crypt ratio was maintained and there was no architectural alteration. Non-specific mild chronic inflammation was observed in 7% of the cases. No case showed any evidence of activity, ulceration, cryptitis or crypt abscess formation. Pyloric gland metaplasia was absent in all. As with the ileum none of the segmental mucosal biopsies from the caecum till the rectum were found to be abnormal. Mild architectural alteration in the rectum and sigmoid was seen in 1 case (3%). 7-30% of the colonic biopsies showed non-specific mild chronic inflammation with a maintained plasma cell gradient. In a study of colonic mucosal biopsies by Seldenrijk et al[50] mild chronic inflammation was observed in 14% of their normal controls. Theodossi et al[51] found 7% of their normal controls to have mild chronic inflammation and 4% to have mild architectural alteration in the rectal biopsy.Levine et al[67] have described the normal histology of the colon and found branching to be quite a common feature at innominate grooves of the rectum.

In our study no case showed any evidence of activity, ulceration, cryptitis or crypt abscesses apart from 1 rectal biopsy that showed mild cryptitis. Our study shows 23% of the rectal/ sigmoid colonic biopsies with mild fibrosis. An explanation for this could the dependent nature of sigmoid colon that acts as a reservoir for faecal matter and bears some amount of shearing force by the same. However none of the cases with fibrosis had any appreciable crypt atrophy or architectural alteration.

The mean mucosal height of the ileal mucosa in our controls was 569.13 µm (±27.2µm) with the mean height of villi being 475.4µm (±31µm).

In the caecum the mean height of crypts and mucosa were 321.8µm(±19µm) and 333.3µm(±21.4µm) respectively. We observed that proceeding distally crypts and mucosal heights increased to 351.53µm(±50.19µm) and 375.07µm(±53.05µm) in the rectum, respectively. In a study by Jenkins et al[54], the mean height of crypts and mucosa in rectal biopsies from normal controls were 373.6 µm and 416.4µm. A Study by Zaitoun et al[96]showed mean crypt and mucosal height for their control subjects to be 481µm(±35µm) and 528µm (±38µm). Thus, our study suggests that the mucosal height in the colon in the Indian population could be less than that of the western population. However, as there is discordance among the western studies themselves, further studies are recommended to confirm these findings.

Our study shows that in the basal part of the mucosa (sub-cryptal and intercryptal lamina propria) plasma cells are normally present. For the ileum, caecum/ascending,

transverse/descending and sigmoid/rectum the mean plasma cell counts under oil immersion field (OIF) were 3.5(±1.5), 6.6 (±1.4), 4.3 (±1.6) and 3.7 (±1.3) respectively. Similarly the mean eosinophil counts at 400X magnification (40X lens = HPF) were 2.3 (±0.8), 5.6 (±1.3), 3.2 (±1.1), 2.6 (±1.2). The findings of our study support the claim that there is an increase in lamina propria cellularity in the right colon as compared with the left colon where the rectum has the least lamina propria cellularity[67,100]. Thus there has to be a substantial increase in plasma cell quantity with a concomitant loss of normal plasma cell gradient for basal plasmacytosis to be considered significant and pathologists should be aware that occasional scattered plasma cells in the basal layer of the mucosa are normal.

CROHN'S DISEASE

Our study showed that the ileum was the site that was most frequently involved in CD (87%) followed by caecum and ascending colon (83%), transverse and descending colon(70%) and the recto-sigmoid (63%) We observed that the degree of involvement was almost most severe in the ileum (mild-27% and moderate/severe -60%) and as we progressed distally the severity and frequency of involvement decreased as evidenced by mild involvement in 40% and moderate involvement in 23% of rectal biopsies. Our findings directly support the study of Bentley et al[86] which showed that there is a decreasing proximal to distal gradient of mucosal changes in CD and this feature played a crucial role in allowing experts to make a correct diagnosis. As the disease severity tapered off in the distal sites, our study also showed that chronic

inflammation followed in parallel. Moderate to severe chronic inflammation was seen in 54% of the biopsies from the ileum that reduced to 47% in caecum and then tapered off to 33% in the rectum and sigmoid colon. Schmitz-Moormann[101] found moderate-severe chronic inflammation in 63% of caecal biopsies, 57% in descending colon and 28.9% in rectum. Though Schmitz-Moorman et al did not have ileal biopsies in their study; the findings for colonic segments are similar.

Our study shows 67% of ileal biopsies in CD have an altered architecture (33% mild and 53% moderate-severe) and63% of rectal biopsies show architectural alteration. These findings differ from those of Geboes et al[102] who showed 84% architectural alteration in ileal biopsies in their study of 88 patients with CD. A study by Seldenrijk et al [50] showed architectural alteration in 71% of rectal biopsies and Theodossi et al showed 65% architectural alteration in their study [51]. These results are similar to our findings.

Like chronic inflammation, activity also appeared to follow a similar fashion being moderate-severe in 30% of biopsies from the ileum, 23% in biopsies from the transverse/descending and 10% in the recto-sigmoid. Geboes et al[102]reported activity in 41% of ileal biopsies while Schimtz-Moormann study had moderate-severe activity in 49.1% of descending colon and 17.6% in the rectum. Our findings are similar. We found crypt abscesses in 10% of ileal biopsies, 27% of transverse/descending colon and 20% of rectal/sigmoid colonic biopsies. Geboes et al did not comment upon crypt

abscesses in their study but Schmitz-Moormann showed crypt abscesses in 22.86% biopsies from transverse/descending and 14.9% of biopsies from recto-sigmoid [101].

Our study showed pseudo-villous change and atrophy in 3% of colonic biopsies which is comparable to the findings of Surawicz et al [73]who reported 6% of cases to have pseudo-villous change and no case to have crypt atrophy. Granulomas were seen in 46.6% (n=14) of our CD cases as reported in other studies[58,103]. 10% of ileal biopsies in our study had granulomas which is concordant with the study by Pulimood et al[92]. It was also interesting to note that 1 case in the study of Makharia et al[33] had necrotising granulomas whereas no case in our study had this feature. Crohn's granuloma as classically described by Lockhart-Mummery, Chambers and Morson and Tandon et al are non-necrotising granulomas[28,58,103]. In the study of Surawicz, Pulimood et al and Das et al no case of CD showed necrotising granulomas[4,19,20,68] .

Our study shows 10% cases have granulomas in the ileum, 23% in caecum/ ascending, 26.6% in transverse/ descending colon and 16.6% in sigmoid-rectum. The study by Schmitz-Moormann[101] had similar findings showing 18.5% of caecum/ascending, 16.5% of transverse/ descending and 12% of sigmoid/rectal mucosal biopsies with granulomas in patients of CD. The study of Pulimood et al [20] showed 5%,17%, 12% and 28% of biopsies with granulomatous inflammation in the ileal, caecum/ascending, transverse/descending and recto-sigmoid mucosal biopsies from patients with Crohn disease.

In our study all cases of CD showed moderate chronic inflammation and/or mild architectural alteration in at least one segment. (65 of 120 sites – chronic inflammation in 46, architectural alteration in 45). Another 6 sites (from 4 cases) showed granulomas so a total of 71 sites showed at least one diagnostic feature of CD.

Correlation with endoscopic findings:

In our study we found moderate/severe chronic inflammation and/or architectural alteration were seen in 49 of 59 biopsies from endoscopically involved segments (41 chronic inflammation; 39 architectural alteration) and 9 of 61 biopsies (chronic inflammation in 5 and architectural alteration in 7) from endoscopically uninvolved segments. Only chronic inflammation without architectural alteration was seen in 9of the involved segments and 2 of the uninvolved. Only architectural alteration was seen in 8 of the 59involved segments and 4 of the uninvolved segments.

Granulomas were seen in 15 of the 59 involved segments (14 cases) and 8 of the uninvolved segments (5 cases - of which in 4, granulomas were only seen in the uninvolved segments). Granulomas were associated with significant chronic inflammation in 1/3, 3/7, 3/8 and 2/5 biopsies from the 4 proximal to distal segments. They were associated with architectural alteration in 3/3, 4/7, 3/8 and 3/5 of the proximal to distal segments. Our study shows that granulomas are often seen in the absence of significant inflammation or architectural alteration suggesting that they could be the earliest detectable histological feature in Crohn’s disease.

Our study shows that morphometric measurements are also concordant with architectural alteration seen histologically as the mean height of ileal villi had a 40% decrease from 475µm in controls to 298µm in the diseased/ involved biopsies of CD cases. There is evidence in the existing literature about architectural alteration that occurs in Crohn's ileitis, however, studies have not quantified and measured its extent in detail, making our study the first in this regard. Similarly ours is also the first study to document and demonstrate the change in the heights of villi and ileal mucosa in Crohn's disease.

In the study of Jenkins et al[54] the mean height of crypts and mucosa in the rectum of chronic CD cases increased from 373.6 µm and 416.4µm to 461.6 µm and 559.2 µm respectively. In the study of Thompson et al [54] the mean height of crypts and mucosa in the rectum of chronic CD cases also increased from 350 µm and 400 µm to approximately 450 µm and 550 µm, respectively. Our study shows that the mean height of crypts and mucosa in Crohn's cases with rectal involvement are 474.7 µm and 527.7 µm (Our study normal rectal crypt height -351.53µm, mucosal height - 375.07µm). This finding of increased height was statistically significant ($p<0.001$). Even though our study has shown possible differences between the height of mucosa between normal controls of Indian and western population, our findings in diseased cases are supported by these western studies displaying similar percentages of increase as compared with the baseline normal controls (Our study – 40.5% increase, Jenkins et al – 34.7% increase, Thompson et al 37.5% increase).

The mean plasma cell counts per oil immersion field in the ileum, caecum/ascending colon, transverse/descending colon and rectosigmoid were similar but were significantly increased as compared to the normal controls ($p<0.001$). These findings are consistent with the loss of plasma cell gradient and basal plasmacytosis as described in literature for IBD. Similarly, the eosinophil count was also increased significantly. An increase of eosinophils was also seen on subjective histological assessment in the study of Geboes et al[102] in ileal biopsies of CD.

Our study showed that there is a 350% increase in plasma cell count for the ileum as compared to the normal controls while it is a 200% increase in the transverse/descending and recto-sigmoid. Even though the right colon has a higher normal count of basal plasma cells, we still had only a 113% increase in plasma cells. Jenkins et al[54] also recorded a significant increase of 122% in rectal biopsies of CD cases in their study. However this figure was for mean cell density in basal 1/3rd of lamina propria that included plasma cells and other cells as well. The increase in the lamina propria of immunoglobulin stained plasma cells was however 270%, which is higher than ours, but then again this count was inclusive of the entire mucosal thickness and not just the basal 1/3rd. No other study has quantified plasma cells in cases of CD in segments other than the rectum. The mean eosinophil count per high power field in the ileum, transverse/descending colon and recto-sigmoid was about 2 fold-higher than in the controls. The increase was less marked in the caecum/ascending colon, where the counts were also higher in the normal controls. Our findings are also supported by other western studies that have observed an increase in eosinophils in cases of CD[104,105]

Ours is the first study in this regard to quantify plasma cells and eosinophils in the entire colon including terminal ileum, documenting statistically significant results in Crohn disease.

INTESTINAL TUBERCULOSIS

Our study shows that the ileum and caecum are the most common histologically affected sites (ileum -83% and caecum -80%). Architectural alteration was seen in 83% of biopsies from the ileum and 70% of caecum/ascending while it tapered down distally to 7% in sigmoid and rectum. Similarly moderate-severe chronic inflammation was highest in the ileum (80%) and then in the caecum/ascending colon (71%) biopsies. No case had any significant inflammation in the rectum with the exception of 1 case that had severe chronic inflammation with ulceration and necrotising granulomatous inflammation in the rectum (Xpert - TB PCR positive case). 67% of biopsies from the ileum and caecum showed deep ulcers and similar frequency was noted for activity. Our study shows 54% and 60% of biopsies from ileum and caecum to have mucosal fibrosis. This is explained by the similar frequency of ulceration in the ileal and caecal biopsies and by the fact that fibrosis occurs secondary to healing. Other studies have shown similar findings in terms of lesions in intestinal tuberculosis to be concentrated around the ileum and caecum[4,20,33].

Our study showed 27% of ileal biopsies had pyloric gland metaplasia. Tandon and Prakash et al have described pyloric gland metaplasia to be common in resection

specimens of ITB. We also observed that none of the mucosal biopsies from cases of ITB had any crypt atrophy or pseudo-villous change. Studies have shown these features to be suggestive of chronic IBD; however their presence in ITB has not been documented. Our study suggests these findings are absent in ITB and can help in ruling out other differentials.

In our study all the cases had granulomatous inflammation and necrotising granulomatous inflammation was recorded in 26% of patients. Other studies have shown variable figures ranging from 13% - 40% in their studies [19,20,31,33,106] and have shown necrotising granulomas to be the strongest discriminator in favour of a diagnosis of ITB. Our study showed granulomas in the ileum were relatively smaller than their counterparts in the colonic segments. The mean granuloma size was larger than 200µm suggesting that a considerable number of granulomas seen in our study were medium sized or large [20]. In the study by Pulimood et al[19] 95% of ITB had at least one granuloma more than 200 µm.

Our study shows that morphometric measurements are also concordant with architectural alteration seen histologically as the mean height of ileal villi had a 60% decrease from 475µm in controls to 183.8µm in the diseased biopsies of ITB cases. As the villi height decreased due to blunting and shortening and the lamina propria expanded due to moderate-severe chronic inflammation, the mean height was similar to the normal controls. In the caecum and ascending colon our study showed statistically significant increase in the mean heights of crypts and mucosa (406µm and

472.7µm respectively) as compared to normal controls. This finding also is in concordance with the histological presence of moderate to severe chronic inflammation commonly seen in the caecum and ascending colon of ITB cases. The heights of crypt and mucosa were equal to the controls in the distal segments.

Our study shows that the mean count of plasma cells/ oil immersion field(OIF) is highest in the ileum and caecum (16 and 20 respectively) and similar to the normal controls in the distal colon and rectum. Similar trend was seen for eosinophils that were higher than their normal counterparts and showed a similar peak in the left colon. These findings suggest that loss of plasma cell gradient, and basal plasmacytosis is not restricted to IBD and ITB must also be added to the list. This fact again reminds us about the difficulty in differentiating ITB and CD on mucosal biopsies alone.

Correlation with endoscopic findings

65 biopsies were from endoscopically involved segments, of which 54 had granulomas. Biopsies from distal segments involved by disease were less likely to show granulomas (3 of 8 from the transverse/descending colon and 2/5 from the recto-sigmoid) compared to biopsies from the proximal segments (25/26 in the ileum and 24/26 in the caecum/ascending colon. Among 55 biopsies from the uninvolved segments, only one showed a granuloma.

ULCERATIVE COLITIS

Our study shows that the rectum and sigmoid are the maximally involved segments in 97% of cases whereas the caecum/ascending colon and ileum are left uninvolved in 27% and 90% of cases, respectively. A study by Robert et al[70] showed the rectum and sigmoid colon to be involved in 100% and 93% of their UC similar to our findings. Their study showed 100% involvement of the transverse, ascending colon and caecum, which is in striking contrast to ours. However their study had a limitation because they had only 6 biopsies from ascending colon and only 1 biopsy from the caecum. The recto-sigmoid biopsies showed moderate-severe architectural alteration and chronic inflammation in 80% and 96% respectively in our study, whereas the caecum/ascending colon and ileum showed significant architectural alteration and chronic inflammation in less than 15% of cases. Activity paralleled chronicity and was significantly seen in 93% of left colonic biopsies as compared to 10% from the right. Our study shows that biopsies from the distal colon have a higher percentage of cryptitis (97%) as compared with the caecum/ascending (50%) and ileum (6%). Paneth cell metaplasia was seen in 30% of biopsies distal to the hepatic flexure and 3% in biopsies proximal to it. Our study showed pseudo-villous change in 13% of recto-sigmoid biopsies. The study by Washington et al[69] showed significant architectural alteration in 86%, chronic inflammation in 76%, activity in 87%, paneth cell metaplasia in 16% and pseudo-villous change in 50% of the rectal biopsies in UC. Theodossi et al[51] also found architectural alteration in 86%, chronic inflammation in 84%, activity in 53%, paneth cell metaplasia in 19% and pseudo-villous change in

30% of rectal biopsies in their study . The findings of both these studies are similar to those of our study.

In our study 70% of recto-sigmoid biopsies had ulceration whereas none of the ileal biopsies had ulcers. Fibrosis was seen more in the left colon as compared with the right (54% in former and 23% in the latter). In our study we also found crypt atrophy to be present in 54% of the biopsies from the left colo-rectum whereas none of the caecum/ascending colonic biopsies had any evidence of crypt atrophy. Highest prevalence of atrophy per se was seen in the recto-sigmoid (47%). Le Berre at al[90] found mucosal ulceration in 100% and crypt atrophy in 43% of rectal biopsies in their study. Theodossi et al[51] found surface ulceration in 40% and crypt atrophy in 44% of their study cases. These findings are also similar and concordant with the findings of our study. Similar distal predominance of disease severity as compared to the right colon was also shown in the study of Bentley et al [86]. Thus, our study reiterates that there is a marked predominance of UC in the recto-sigmoid and that the disease decreases in its severity as one moves proximally towards the ileum, but moreover we have quantified the subjective histological parameters with numerical figures for the entire colon and terminal ileum.

On morphometric measurements the mucosal height in the ileum and caecum had no significant change and are similar to those of the normal controls. This finding is in concordance with the paucity of changes seen on histology in the proximal segments. Our study shows a 50% increase in mucosal height in the recto-sigmoid with a

concomitant 900-1000% increase in mean plasma cell count/ OIF and a 50% increase in mean eosinophil count/HPF. These findings directly reflect the degree of architectural alteration, chronic inflammation and basal plasmacytosis in UC.

Jenkins et al also showed a 25% increase in mucosal height and Zaitoun et al showed a 35% increase in their studies on rectal mucosal biopsies in UC patients [54,96]. The study by Jenkins et al showed a 90% increase in cellularity in the basal 1/3rd of mucosa, but again, this figure was for mean cell density that included plasma cells and all other inflammatory cells as well. The increase in the lamina propria of immunoglobulin stained plasma cells was however 260% but again this count was inclusive of the entire mucosal thickness and not just the basal 1/3rd. No other study has quantified plasma cells or measured the heights of mucosa and crypts or quantitated histological parameters in cases of UC other than the recto-sigmoid. Our findings suggest that quantitation and morphometric analysis when used as adjuncts to histological assessment are useful tools.

Inter-observer correlation

The inter-class correlation coefficient (ICC) for inter-observer variability was good (for plasma cell counts it was 0.93 (range 0.86 to 0.96) and for eosinophils was 0.87 (range 0.81 to 0.94). This was probably because plasma cells and eosinophils are easily identifiable in tissue sections that are stained with haematoxylin and eosin.

DIFFERENTIATION OF CROHN'S DISEASE FROM ULCERATIVE COLITIS (CD vs. UC)

I. Quantification of Histological Features.

Ileum

Our study shows that the ileum is significantly involved in CD when compared to UC. UC cases showed only mild involvement of the ileum in 10% of biopsies whereas CD had 87% involvement (27% - mild and 60% - moderate to severe). 54% of our CD cases had significant chronic inflammation and 67% showed significant architectural alteration in the ileum. In striking contrast was UC, with a mere 14% and 17% of ileal biopsies showing significant chronic inflammation and architectural alteration. Our study shows significant activity and cryptitis in 74% and 37% of ileal biopsies in CD, whereas only 17% and 7% of UC cases showed the same. 30% of our CD cases had ulceration on histology in the ileal biopsies whereas none of the UC cases had any ulcers in the ileum. Geboes et al[102] studied ileal biopsies of CD and found architectural alteration in 84%, cryptitis in 41% and pyloric gland metaplasia in 2% of their study cases. They suggested that the paucity of inflammation and architectural involvement of the ileum is more in favour of UC than CD. We concur with their findings as UC cases in our study also showed a paucity of involvement and inflammation in the ileal biopsies. A study on resection specimens of UC by Haskell et al[47] found overall incidence of ileal involvement in UC to be 17% and mild in nature, similar to the findings of our study. Other contemporary studies have shown

the ileum when involved in UC, has an incidence ranging from 5% to 10%[107–109]. Our findings are similar to these studies in this regard.

In our study we found 17% of the CD cases had pyloric gland metaplasia in the ileum, whereas one of the UC (3%) case showed this feature. Tanaka et al [110]considered the presence of pyloric gland metaplasia as evidence of CD over UC in their study [110].A study by Koukoulis et al[62] showed pyloric gland metaplasia in 22.2% of ileal biopsies in patients of CD. Our findings are also similar to those of Goldstein et al[63] who showed pyloric gland metaplasia in 27% of ileal biopsies from CD patients and 10% of ileal involvement in UC cases. In our study 3 cases of CD had granulomas in the ileum (10%) whereas none of the UC cases had granulomas. The role of granulomas in differentiating CD and UC has already been extensively described in literature.

Colon and Rectum

In our study we saw that the caecum/ascending colon (right colon) was involved in 83% of CD cases compared to 30% of UC. In the caecum/ascending colonic biopsies the features that are significantly higher in CD as compared to UC are architectural alteration (54% vs. 27%), moderate-severe chronic inflammation (47% vs. 10%) and activity (70% vs. 53%). Deep ulceration in the caecum was seen in 14% of the cases of CD as compared to 3% of UC. In the transverse/ descending colon, UC cases showed a higher frequency of activity, cryptitis, Paneth cell metaplasia and pseudo-

villous change than cases of CD. None of our CD cases showed Paneth cell metaplasia or pseudo-villous architecture in the transverse/descending colonic biopsies. Our study showed an interesting reversal of severity in the distal colon. Unlike the caecum and ascending colon where CD had significantly higher frequency for all the histological features, UC had a significantly higher frequency for all the histological features in the rectum and sigmoid. UC involved the left colon in 97% of the biopsies whereas CD involved this site in 63%. Our frequency of 37% rectal sparing is in keeping with the findings of other studies[110]

The changes in the sigmoid-rectum showed significant difference among these 2 forms of chronic IBD. All the rectal biopsies from UC cases in our study (100%) had architectural alteration compared to 30% in CD cases. Furthermore, the extent of architectural alteration was moderate-severe in 80% of UC and only 14% in CD cases. Our study shows that 96% of rectal biopsies from rectum/ sigmoid have moderate-severe chronic inflammation compared with 33% of CD. The presence of focally enhanced colitis was observed in 17% of CD whereas none of the UC cases showed this feature. Cryptitis and activity were seen in 96% and 97% of biopsies in UC compared to 53% and 10% in CD, respectively. In our study 70% of recto-sigmoid biopsies showed ulceration in UC cases whereas no case of CD showed ulceration at this site. The study by Seldenrijk [50]showed architectural alteration in 92% of UC cases and Le Berre et al's[85] study showed this feature in 95% of UC cases. Our findings are similar. As there was a higher incidence of ulceration in UC the incidence of fibrosis was also higher as compared to CD cases (54% vs 37%) in our study. Our study

shows no significant difference in crypt distortion and branching in left colon to differentiate among CD and UC. However, in the right colon UC has significantly more crypt distortion compared to CD (20.7% vs. 5.1%). In our study UC shows significantly higher crypt branching than CD in the left side of colon and rectum (4.8% vs. 1.3%). More than two crypt abscesses in a site were seen in 12 biopsies from the transverse/descending colon (40%) and 23 biopsies from the rectosigmoid (77%) in UC, but only 1 from the caecum/ascending colon 2 from the recto-sigmoid in CD.

Of note was the presence of pseudo-villous change and crypt atrophy in cases of UC that were seen in 14% and 47% of recto-sigmoid biopsies respectively. These 2 features were consistently absent in all the sigmoid/ rectal biopsies of CD cases in our study. These findings are supported by the works of Seldenrijk et al[111] whose study showed pseudo-villous change in 17% of UC cases and none of CD. Theodossi et al's[51] study also showed pseudo-villous change and crypt atrophy in 30% and 44% of UC cases though it was also seen with a significantly lower frequency in cases of CD. Le Berre et al[90] had peudo-villous change in 88% and crypt atrophy in43% of rectal biopsies in their UC cases. Cook and Dixon[60] documented 100% architectural alteration and 78% crypt atrophy in the rectal biopsies of UC as compared to 68% and 36% in CD of their study. Thus our findings are again similar to those of western studies. We can thus infer that a distal colonic/rectal biopsy in UC is significantly more likely to show villous mucosal architecture and crypt atrophy as compared to

CD. Other investigators have further confirmed the reproducibility and reliability of these features as highly discriminant through various studies which are in support of our findings[68]. However, ours is the first study that has found statistically significant differences to differentiate UC from CD with biopsies from the left colon in addition to the right side colon. Our study confirms that CD has a relative decrease in the disease severity in the distal colonic segments whereas UC increases in its severity in the distal colonic segments. This significant trend that emerged in our study is in direct agreement with those of Bentley et al[86]. In our study 36% (n=11) of CD cases showed granulomas in the colonic segments while none of the UC cases showed any granulomas. Comparable frequencies of granulomas in the colon have also been observed by other studies that attempted to differentiate CD from UC in sigmoid and rectal mucosal biopsies[50,68,85,91].

II. Morphometry

Ileum

Our study shows a significant difference in the ileal villous height of cases with CD and UC. The mean villous height in involved cases of CD is 298µm whereas it is 422.8µm in UC. The villi in CD have shown 40% reduction in height as compared to normal controls as a result of significant architectural alteration whereas in UC our study did not show significant architectural alteration and this correlates with the mean height of villi being comparable with those of our normal controls. This significant reduction in height is corroborated by the presence of architectural

alteration on our histological assessment and serves as numerical evidence to our subjective estimation of villous atrophy. It is interesting to note that the mean mucosal height in the ileum were similar in both diseases and controls. This phenomenon can be explained due to the inflammatory infiltrate that expands lamina propria causing a "compensatory increase" for the shortened villi and thus creating a relative increase in the net mucosal height. As ours is the first study to measure these parameters, we suggest further studies be carried out to confirm our findings before their use in routine diagnostics.

Colon and Rectum

In our study, cases of CD and UC which involved the caecum/ascending colon were found to have similar increase in the average heights of crypts and the mucosa. The increase in mucosal heights was statistically significant when compared with normal controls. The same trend was also observed in the transverse/descending colonic biopsies for both the diseases.

In the rectum, we observed the average height of mucosa to be 527µm for CD and 578µm for UC. Thus, there was a significant 40% increase in mucosal height for CD and a significant 54% increase in mucosal height for UC as compared with normal controls, pathologically this increase in mucosal height was not found to be helpful in differentiating these 2 diseases. In the study of Zaitoun et al[96] a comparable 40% increase in height of untreated UC cases was shown. A morphometric study by Thompson et al[97] on rectal biopsies also showed a similar 40% increase in mucosal height for CD and 62% for UC cases. Though mucosal height was significantly higher

in UC and CD when compared with normal controls, it failed to differentiate between UC and CD. This is similar to our finding. In the study of Jenkins et al[54] also the increase in mucosal height was not statistically different to distinguish between CD and UC. Their CD cases had a 35% increase in height while UC cases had a 25% increase. Our findings differed from this study only in this regard.

In our study we observed that for CD in the rectum/sigmoid, the difference between the height of mucosa and crypts was 53µm whereas for UC cases in this site the difference was 114 µm. This was due to the higher frequency of atrophy that we found in cases of UC as compared with CD. Studies have proved that crypt atrophy in the recto-sigmoid region is a highly discriminant feature of UC as compared with CD and our study supports their findings in addition to providing numerical evidence for the same[50,51,68,85,86]. When we selectively measured the mean heights of crypt and mucosa of UC cases with atrophy seen on our histological examination, we found the difference to be 189 µm.

III. Cell Counts

Ileum

In our study we found that for CD, the basal part of involved ileal mucosa shows a 350% increase in the average plasma cell count/OIF. Our study shows no increase of plasma cell count/ OIF in the basal part of ileal mucosa in UC. We also observed a

200% increase in eosinophil count/ HPF in CD compared to the normal controls. An increase of eosinophils was also seen in the study of Geboes et al[102] in ileal biopsies of CD. Other western studies have also observed an increase in eosinophils in cases of CD[104,105].

Colon and Rectum

In caecum / ascending biopsies we found UC to have a significant 200% increase in basal plasma cell count whereas CD had a relatively lower 100% increase in basal plasma cell count. Again CD had a higher 60% increase in eosinophil count compared to 25% in UC cases. However, in the left side of the colon (transverse/descending colon and recto-sigmoid) the increase in plasma cells at the base of mucosa for our UC cases was exponentially higher. Our study showed a 600% and 1000% increase in basal plasma cell counts for UC cases whereas for CD cases the increase was 200% and 220% respectively. These findings are statistically significant and are direct numerical evidence of the fact that in sigmoid and rectal biopsies the basal plasmacytosis and chronic inflammation is more severe in UC than CD, as found in other western studies[54,66,68,85,86,94]. The observed increased percentage of eosinophils in recto-sigmoid biopsies was similar and in both UC and CD, and was not found to be helpful as a discriminant finding. A study by Rosekrans et al[77] showed the increase of IgM plasma cells in the lamina propria to be suggestive of CD in sigmoid and rectal biopsy specimens. They also showed that an increase of IgG plasma cells was suggestive of UC from the same biopsy sites. A study by Skinner et al[78] on colectomy

specimens showed an increase of IgA plasma cells to be more suggestive of CD than UC. However, later studies by Scott et al[94] and Jenkins et al[54] showed that though there was a significant increase of plasma cells in the lamina propria, the number could not differentiate between CD and UC. These western studies have at least proven that

1. In chronic IBD there is a significant increase of the plasma cell count in lamina propria.

2. There is loss of normal plasma cell gradient within the mucosa.

However, these studies counted plasma cells in the entire thickness of the mucosa. Jenkins et al[54] showed that the ratio of plasma cells in the upper half of the mucosa is 1.8:1 times that of the lower half in normal controls. In our study we have exclusively counted the plasma cells in the lower part of the mucosa i.e. the inter-cryptal and sub-cryptal region under oil immersion field (OIF). Thus, as our methodology and study design differs from these studies, so do our findings. Though to an extent our findings are similar, with respect to the significant increase of lamina propria plasma cells, our study shows that plasma cell count in the lower-most portion of mucosa helps in differentiation between UC and CD.

DIFFERENTIATION OF CROHN'S DISEASE FROM INTESTINAL TUBERCULOSIS (CD vs. ITB)

I. Quantification of histological features

Ileum

Our study shows that the ileum is involved in equal frequency by both CD and ITB, but significant differences emerged in the amount of inflammation and architectural alteration. Tandon and Prakash[28] also demonstrated that the ileum and ileo-caecal segment are the most commonly involved sites for both ITB and CD. In our study ITB had a significantly higher frequency of architectural alteration (83%) and moderate-severe chronic inflammation (80%) as compared to Crohn (67% and 53% respectively). In a previous study from our institute, moderate to severe chronic inflammation (ileal and colon cumulative) was seen in 73% of ITB compared to 47% in CD, which is also similar to our findings in the present study. In a recent Singaporean study, Kumarsinghe et al[112] described histological changes in endoscopic mucosal biopsies of CD and have shown ileum to be involved in 96% of their cases which is in concordance with the 87% of our present study. Their study shows features of chronicity to be81% that is similar to the 87% of our study. Similarly Geboes et al[102] have also observed features of chronicity in 84% of ileal biopsies of CD in their study. In our study, the frequency of pyloric gland metaplasia in the ileum was slightly higher in ITB than CD (27% and 17%).Our finding is supported by the

study of Yokoyama et al[113] that shows a similar higher frequency of pyloric gland metaplasia in resected ileal specimens of ITB as compared to CD (84% and 65%) .

Our study shows that ileal Tuberculosis is 2 times more likely to show deep ulceration (67% vs 30) on histology, fibrosis in the lamina propria (54% vs 26%) and cryptitis (64% vs 37%) as compared with CD. To the best of our knowledge no study has quantified and characterised the histological changes of ileal biopsy in similar detail to differentiate CD from ITB, making our present study possibly, the first in this regard. We believe that these findings can be of vital aid to a pathologist plagued with the diagnostic dilemma to differentiate between ITB and CD; that is becoming increasingly common nowadays.

In our study 10% cases of CD had granulomas in the ileum whereas 83% of ITB cases showed granulomas in this site. In CD the maximum dimension of granuloma was 95µm (mean 65.6 µm) whereas in ITB the largest granuloma in the ileal biopsies was 440µm (mean 208µm). This shows that in ileal biopsies the granulomas of Tuberculosis are significantly larger in size as compared to those in CD. In the ileum, our study shows necrotising granulomas in 5 cases of ITB cases whereas none of the granulomas of CD showed necrosis.

Colon and Rectum

Our study shows that the caecum and ascending colon are also involved in equal frequency by both CD and ITB, but with significant differences as observed for the ileum. ITB had a significantly higher frequency of architectural alteration (70%) and moderate-severe chronic inflammation (70%) as compared to CD (54% and 47%). In our study 67% of the ITB cases showed ulceration whereas only 13% of the CD cases showed ulcers. Similarly, fibrosis in the lamina propria was observed in 60% of ITB cases whereas 17% of CD cases showed this feature. In our study 7% of the caecal/ascending colon mucosal biopsies of CD cases showed pyloric gland metaplasia whereas none of the ITB cases showed this feature. Our finding is supported by the study of Yokoyama et al[113] that also did not show pyloric gland metaplasia in any of the resected specimens of colonic TB. Previous published studies from our institute and studies by other authors have shown that CD has a significantly longer duration of illness compared to a shorter duration of illness in ITB[9,19,33]. This can explain possibly explain why pyloric gland metaplasia (a reliable feature of chronicity), in the caecum is far more common in CD as compared to ITB.

Our study shows a remarkable decrease in the severity of disease in ITB as we progress distally towards the rectum. The transverse/ descending colon were involved in 30% of cases whereas the sigmoid/rectum was involved in 7% of the cases. In sharp contrast lies CD involving the transverse colon in 70% of cases and the sigmoid/rectum in 63% of cases. This distribution of involvement of CD is concordant

with the study by Schmitz-Moormann et al[101] and has been discussed in the previous section of CD.

In the rectum/sigmoid, our study shows significant difference between CD and ITB in terms of architectural alteration (30% CD vs 6%, ITB, $p<0.05$) and chronic inflammation (64% CDvs. 13% ITB, $p<0.05$). Schmitz-Moormann et al[101]found chronic inflammation in 73% of recto-sigmoid biopsies in their study on adult CD cases. Le Berre et al[90] and Surawicz et al[68] also showed a similar frequency of chronic inflammation in their study of CD cases on rectal biopsies. In another study on rectal biopsies by Surawicz et al, architectural alteration was shown by 26% of CD cases. All these western studies are similar to our findings in the present study. Our study shows that cryptitis is 7 times more common in CD than ITB (54% vs. 7%) in the recto-sigmoid and lamina propria fibrosis is 3 times more common in CD as compared to ITB in the recto-sigmoid. Although Makharia et al[33] found both CD and ITB cohorts in their study to have similar frequencies of architectural alteration, chronic inflammation and cryptitis; in our present study we found statistically significant difference among CD and TB for these parameters in the rectal-sigmoid biopsies. We believe as we have segregated the anatomical sites and studied each parameter in each site individually, our results compared with theirs are relatively different. Our findings in the proximal colon and transverse/descending colon are comparable though. Western studies on CD with regard to sigmoid and rectal mucosal biopsies on CD report findings similar to our present study[68,73,85,101].

In our present study “focally enhanced colitis” was seen 2 times more frequently in CD as compared to ITB. Pulimood et al[19] and Makharia et al[33] also found focally enhanced colitis to be significantly more common in CD as contrast to ITB. None of the cases of CD or ITB showed any evidence of pseudo-villous change or crypt atrophy in our study`.

Our study supports the findings of Tandon et al who also showed that the majority of disease pathology in ITB is confined to the terminal ileum and ileo-caecal region unlike that of CD[28].

In our study, the distribution of granulomas within different sites of the colon was strikingly different between ITB and CD. Granulomas were seen in 83%, 16% and 6% of biopsies from caecum/ ascending colon, transverse/descending colon and recto-sigmoid. In Crohn’s disease our study showed granulomas in 26%, 30% and 16% of biopsies from caecum/ ascending colon, transverse/descending colon and recto-sigmoid. Our study shows that the mean dimension of granulomas in the colon and rectum is 268 µm (range 95 to 525µm) for ITB whereas for CD the mean size of granulomas is 88µm (range 60 to 145 µm).The proposal to measure the number and size of granulomas per section to differentiate CD from ITB has gained wide acceptance in literature and has been corroborated by other studies. A study by Pulimood et al in 1999 has shown that 95% of granulomas in CD are less than 200

µm. In a recent study Makharia et al[33] supported their findings by showing 90% of granulomas in CD were less than 200µm in their study. In our present study none of the granulomas in CD were larger than 200 µm.

Furthermore, our study shows 37% of ITB granulomas are more than 400µm in dimension. Our findings are concordant with the study of Pulimood et al[20] that showed 51.5% of ITB granulomas to be greater than 400µm. A study by Das et al[106] also showed that 50% of ITB granulomas were greater than 400µm. Our study and these studies provide further evidence and support to the role of measuring granuloma size to differentiate between CD and ITB**.** Necrotising granulomas were seen in 26% cases of ITB (n=8), whereas necrotising granulomas were not seen in any of the CD cases (n=0) in our study These findings are in concordance with the existing literature[19,20,28,30–32,103,106].

Study by Das et al[106] shows 40-45% of ITB cases have more than 5 granulomas/biopsy site whereas none of the CD case showed this feature. Study by Makharia et al[33] shows 22.2% of ITB cases have more than 5 granulomas/biopsy site compared with 2 cases of CD which showed this feature. Das et al and Makharia et al have confirmed both confirmed the findings of the original study by Pulimood et al[19] that showed 45% of ITB cases had more than 4 granulomas/site whereas none of the CD case shows this feature. In our present study 30% (n=9) cases of ITB show >4 granulomas per biopsy site whereas none of the cases of CD had more than 4

granulomas per biopsy site. Our finding provides further evidence in favour of using this feature as a discriminant feature in differentiating ITB from CD.

II. Morphometry

Ileum

Our study shows a significant difference in the ileal villous height of cases with CD and ITB. The mean villous height in involved cases of CD is 298µm whereas it is 184µm in ITB. The villi in CD show a 40% reduction in height whereas in ITB the villous height has a 60% decrease. This significant shortening of villi directly correlates with the finding of higher frequency of architectural in cases of ITB as compared with CD serving as numerical evidence for our histological assessment.

Though our study shows statistically significant difference in the mean height of ileal mucosa between CD and ITB, it is not a highly appreciable feature on histological evaluation as one's eye automatically goes to the architectural alteration, chronic inflammation and the enigmatic granulomas that are far more conspicuous, depending upon the individual case.

Colon and Rectum

Our study shows that there is no significant difference between the height of crypts and mucosa of CD and TB case as both were increased in almost a similar fashion.

Though our study showed significant difference in the incidence of architectural alteration and chronic inflammation between ITB and CD at this site, this was not reflected in the mucosal heights. One reason could be that fibrosis was more common in ITB than CD, and could have been responsible for a decrease in the mucosal height, although there was no direct correlation between the presence of fibrosis and alteration in the mucosal height In the transverse/descending our study shows significant difference between mucosal heights of CD and ITB (487µm vs. 375µm). In the rectum there is no increase in the average mucosal height of ITB cases, but cases of CD show a 40% increase in mucosal height. This 40% increase of rectal mucosal height in CD is supported by western studies[54,97]. Morphometric changes of mucosa in cases of tuberculosis have not been studied so far, making our study the first of its kind in this regard. In our study we have measured a few morphological parameters of the mucosa and quantified the histological features in a detailed and comprehensive format taking each anatomical segment from the terminal ileum and the entire length of colon till rectum to search for differences between CD and ITB.

III. Cell Counts

Ileum

In our study we found CD cases to have a 350% increase in average plasma cell counts/OIF in the basal part of involved ileal mucosa. However in ITB cases also there was significant basal plasmacytosis and loss of plasma cell gradient, a feature that is regarded as a highly reliable and discriminant feature of IBD[65,71,73,75,114]. This

fact was reflected in the counts also. Our study shows ITB cases have a 460% increase in average plasma cell count/ OIF in the basal part of involved mucosa that was significantly higher than what our study shows for CD.

In our present study we found CD to have a significantly high (200%) increase in eosinophil counts/HPF as compared to 90% in ITB cases in ileal biopsies. In the study of Geboes et al[102] also eosinophilia was seen in the ileal biopsies of cases with CD whereas none of their controls showed any appreciable eosinophilia. Other investigators have also shown a higher percentage of eosinophils in cases with CD.

Colon and rectum

In our present study caecum and ascending biopsies of ITB had a significantly higher frequency of architectural alteration and moderate-severe chronic inflammation (70%) as compared to CD (47%) and the same was reflected in the cell counts.

Our study shows a 250% increase in basal plasma cell counts in the caecum/ascending colonic biopsies from cases of ITB that is significantly higher the 115% increase seen in CD cases. However, the increase in the eosinophil count/HPF was approximately 2 times higher in CD than TB (55% vs. 30%).

Our study shows a decrease in involvement of the transverse/descending colon by ITB cases as compared to CD and we observed a correspondingly higher (200%) increase

in plasma cell count/OIF in CD cases and a significantly lower increase of 100% in ITB cases. The mean eosinophil count/HPF of ITB is similar to the normal controls throughout the left colon and rectum, whereas CD shows a 100-150% increase in mean eosinophil count/ HPF in the descending colon and recto-sigmoid biopsies. Our study shows a 220% (significant) increase in basal plasma cell count/OIF of CD cases in biopsies from the recto-sigmoid.

Our study shows significant differences in the ileal and segmental colorectal mucosal biopsy specimens at the histological, morphometric and cell count level that, used in the correct clinical, radiological and endoscopic context, can be complementary tools in differentiating Crohn’s disease, intestinal tuberculosis and Ulcerative colitis from one another.

CONCLUSIONS

- Our study of ileal and colonic mucosal biopsies from normal controls has shown that they are similar to mucosal biopsies from normal controls in the West. This suggests that tropical enteropathy and colopathy may not currently be as widely prevalent in India as has been previously reported.

- The mean eosinophil count per HPF in the ileal and colonic mucosa of individuals in India has been documented for the first time (2.3 in the ileum, 5.6 in the proximal colon, 3.2 in the mid colon and 2.6 in the distal colon). They are similar to those reported from the West.

- The morphometric features and histological findings of CD and UC are similar to those reported from the West, suggesting that disease patterns are identical.

- The inflammatory change in CD showed a proximal to distal decrease in intensity and those in UC a proximal to distal increase. ITB predominantly involved the ileum and caecum/ascending colon.

- The prevalence and intensity of involvement of the ileum and caecum as reflected in the presence of deep ulcers, architectural alteration, significant chronic inflammation, plasma cell counts and activity were higher in ITB than CD.

- The granulomatous inflammation in ITB was significantly more intense than that in CD with larger granulomas and higher numbers per biopsy site. In addition, the granulomatous inflammation in ITB was almost always (28 of 30 cases) associated with deep ulceration in the same site and frequently associated with a high plasma cell count/ OIF in the deep mucosa (> 20 plasma cells/ OIF in the deep mucosa in 17/30 cases). In CD, only 2 cases showed

deep ulceration in the same site as granulomas and 2 showed more than 20 plasma cells/ OIF in the sites with granulomas. This suggests that granulomas are an early feature of the pathology of CD whereas in ITB they are part of a more intense mucosal pathology.

- In support of the above finding, granulomas were found in 8 sites that did not show any endoscopic involvement by disease in CD, but only in 1 endoscopically uninvolved site in ITB.

- No case of CD had a granuloma >200μm or >4 granulomas/site.

- The percentage of distorted crypts in diseased mucosa was highest in UC, then in CD and lowest in ITB.

- Rectal involvement or sparing is a feature classically used to differentiate UC from CD. We found that it could also be useful in the differentiation of CD from ITB. None of the ITB cases had more than 10 plasma cells/ OIF in the rectum, except for those that showed granulomatous inflammation.

- Crypt abscesses are significantly more frequent in UC than in CD or ITB.

- Pyloric metaplasia was most common in TB and Paneth cell metaplasia was most common in UC.

- Pseudo-villous change and crypt atrophy were seen predominantly in UC.

- Fibrosis was more frequent in TB than CD. It was most frequent in the distal colon in UC.

- The ileal mucosal height does not change significantly in CD or ITB, but the colonic mucosal height does increase in CD, ITB and UC. The increase was most severe in the distal colon in UC.

- Mucosal plasma cell counts were highest in the mid and distal colon in UC (29 and 42/ OIF) and then in the caecum in ITB.

- In transverse/descending colon and recto-sigmoid biopsies UC shows a 600% and 1000% increase in the mean basal plasma cell count/ OIF.

- Basal plasmacytosis is more severe in ITB than CD and cannot be used to differentiate them.

- Mucosal eosinophil counts were highest in the ileum (6.5 per HPF) and caecum (8.7 per HPF) in CD.

ANNEXURE

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA.

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Alfred Job Daniel, D Ortho, MS Ortho, DNB Ortho
Chairperson, Research Committee & Principal

Dr. Nihal Thomas,
MD., MNAMS., DNB (Endo), FRACP (Endo), FRCP (Edin), FRCP (Glasg)
Deputy Chairperson
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

April 28, 2015

Dr. Tanush Vig
PG Registrar
Department of General Pathology
Christian Medical College, Vellore 632 004

Sub: **Fluid Research Grant Project:**
A morphometric study on intestinal mucosal biopsies in inflammatory bowel Disorders.
Dr. Tanush Vig, Dr. Anna Pulimood, General Pathology, Dr. Sudipta Dhar Chowdhury, Dr. A. J. Joseph, Ms. Mahasampath Gowri S, Biostatistics, CMC, Vellore.

Ref: IRB Min No: 9546 [OBSERVE] dated 03.03.2015

Dear Dr. Tanush Vig,

I enclose the following documents:-

1. Institutional Review Board approval 2. Agreement

Could you please sign the agreement and send it to Dr. Nihal Thomas, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board

Dr. NIHAL THOMAS
MD MNAMS DNB(Endo) FRACP(Endo) FRCP(Edin) FRCP(Glasg)
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

Cc: Dr. Anna Pulimood, General Pathology, CMC, Vellore.

1 of 4

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002.
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PROFORMA

A MORPHOMETRIC STUDY ON INTESTINAL MUCOSAL BIOPSIES IN INFLAMMATORY BOWEL DISORDERS

4 copies: Ileum, caecum/ascending colon, transverse/descending colon, Sigmoid/rectum.

Biopsy number : xxxxx/ yy

Age: **Gender** :

Endoscopic finding:

MORPHOMETRY

Mucosal and crypt height :

	1	2	3	4	5	Mean
Mucosal height						
Crypt height						
Villous height						

Number of crypts in biopsy:

Number of crypts in diseased area:

Number of distorted crypts :

Number of branched crypts :

Number of Plasma cells/Oil immersion field(OIF) and Eosinophils (HPF) in the deep part of the mucosa

	1	2	3	4	5	Mean
Number of plasma cells/ OIF						
Number of eosinophils/ HPF						

Granuloma size:

Granuloma serial number	Maximum diameter	Necrotising/ non-necrotising

HISTOLOGICAL STUDY

Chronic inflammation : Mild / Moderate / Severe

Architectural alteration : Mild / Moderate / Severe

Cryptitis : Mild / Moderate / Severe

Focal activity: Yes/ No.

Activity: Mild / Moderate / Severe

Crypt abscesses: 1-2 , 3-5, >5

Granuloma: Yes / No

If Yes : Necrotizing / Non-necrotizing granulomatous inflammation

Metaplasia : Pyloric gland metaplasia/ Paneth cell metaplasia

Deep ulcers: Yes/ No

Fibrosis: Yes/No

Pseudo-villous change: Yes/No

Atrophy: Yes/ No

Involved segment: Yes/ No (Mild/ moderate/ Severe)

NOTES (if any):

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